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Prenatal Environmental Exposure and Neurodevelopmentally Important Gene Expression in Malformed Brain Tissue from Pediatric Intractable Epilepsy Patients

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PRENATAL ENVIRONMENTAL EXPOSURE AND NEURODEVELOPMENTALLY IMPORTANT GENE EXPRESSION IN MALFORMED BRAIN TISSUE FROM PEDIATRIC INTRACTABLE EPILEPSY PATIENTS

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To: Interim Dean Michele Ciccazzo
Robert Stempel College of Public Health and Social Work

This dissertation, written by Brenda Luna, and entitled Prenatal Environmental Exposure and Neurodevelopmentally Important Gene Expression in Malformed Brain Tissue from Pediatric Intractable Epilepsy Patients, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: July 13, 2011

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DEDICATION

For Cielito and Hector

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I thank my parents, Cielito and Hector, for all of their support and positivity. I am thankful to Anthoni and my family for all of their encouragement.

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ABSTRACT OF THE DISSERTATION

PRENATAL ENVIRONMENTAL EXPOSURE AND NEURODEVELOPMENTALLY IMPORTANT GENE EXPRESSION IN MALFORMED BRAIN TISSUE FROM PEDIATRIC INTRACTABLE EPILEPSY PATIENTS

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The primary objective of this proposal was to determine whether mitochondrial oxidative stress and variation in a particular mtDNA lineage contribute to the risk of developing cortical dysplasia and are potential contributing factors in epileptogenesis in children. The occurrence of epilepsy in children is highly associated with malformations of cortical development (MCD). It appears that MCD might arise from developmental errors due to environmental exposures in combination with inherited variation in response to environmental exposures and mitochondrial function. Therefore, it is postulated that variation in a particular mtDNA lineage of children contributes to the effects of mitochondrial DNA damage on MCD phenotype. Quantitative PCR and dot blot were used to examine mitochondrial oxidative damage and single nucleotide polymorphism (SNP) in the mitochondrial genome in brain tissue from 48 pediatric intractable epilepsy patients from Miami Children's Hospital and 11 control samples from NICHD Brain and Tissue Bank for Developmental Disorders.

Epilepsy patients showed higher mtDNA copy number compared to normal health subjects (controls). Oxidative mtDNA damage was lower in non-neoplastic but higher in neoplastic epilepsy patients compared to controls. There was a trend of lower mtDNA oxidative

damage in the non-neoplastic (MCD) patients compared to controls, yet, the reverse was observed in neoplastic (MCD and Non-MCD) epilepsy patients. The presence of mtDNA SNP and haplogroups did not show any statistically significant relationships with epilepsy phenotypes. However, SNPs G9804A and G9952A were found in higher frequencies in epilepsy samples. Logistic regression analysis showed no relationship between mtDNA oxidative stress, mtDNA copy number, mitochondrial haplogroups and SNP variations with epilepsy in pediatric patients. The levels of mtDNA copy number and oxidative mtDNA damage and the SNPs G9952A and T10010C predicted neoplastic epilepsy, however, this was not significant due to a small sample size of pediatric subjects. Findings of this study indicate that an increase in mtDNA content may be compensatory mechanisms for defective mitochondria in intractable epilepsy and brain tumor. Further validation of these findings related to mitochondrial genotypes and mitochondrial dysfunction in pediatric epilepsy and MCD may lay the ground for the development of new therapies and prevention strategies during embryogenesis.

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CHAPTER I

INTRODUCTION

Overview

Epilepsy is a chronic neurological disorder, characterized by spontaneous recurring seizures. The incidence of epilepsy is highest for children and the elderly. A proportion of patients have seizures that are resistant to traditional anti-epilepsy medicines (intractable or refractory epilepsy) (Alexander and Godwin, 2006). The occurrence of epilepsy is highly associated with malformations of cortical development (MCD), which are developmental brain lesions that consist of dysplastic neuronal lesions (malformations) (Schwartzkroin and Walsh, 2000; Hua and Crino, 2003; Hader et al., 2004; Rickert, 2006; Wong, 2007). MCD are increasingly being recognized as the cause of intractable epilepsy. MCD presents a broad spectrum of structural changes which appear to result from changes in precursor neuronal or neuronal cells during cortical development at various stages such as: proliferation, migration, differentiation, and apoptosis (Becker et al., 2004).

Treatment with anti-epileptic drugs (AEDs) is usually ineffective, and children with MCD may require surgical removal of the affected area of the brain (Yasin et al., 2010). In surgical series, focal cortical malformations and low-grade tumors (gangliomas, gangliocytomas, dysembryoplastic neuroepithiliomas, and astrocytomas) (Saneto and Wyllie, 2000). Two of the most common MCD found in resected tissue from children afflicted with intractable epilepsy are tuberous sclerosis (TSC), and focal cortical dysplasia (FCD) (Fassunke et al., 2004). A significant proportion of FCD patients are not

appropriate surgical candidates or continue to have seizures despite the surgery. The molecular mechanisms underlying the formation of MCD are still largely unknown and the treatments for epilepsy due to MCD are often ineffective or limited (Wong, 2009). Therefore, MCD formation and the occurrence of epileptic seizures in children is a great public health concern.

Several reports indicate prenatal events are likely to be involved in the pathogenesis of MCD (Montenegro et al., 2002). Since brain development commences early in fetal life and continues until adolescence, exposure to environmental chemicals at an early stage may be the leading cause of neurodevelopmental disorders (Allen and Walsh, 1999). Several studies indicate that genetic and environmental factors contribute to causation of MCD and lead to neurodevelopmental delay (Kuzniecky and Barkovich, 2001). Human and animal studies have demonstrated that *in utero* exposure to environmental agents such as: chemical (ethanol), physical (ionizing radiation) and biological factors (toxoplasmosis) result in neuronal migrational disorders (Chevassus-au-Louis et al., 1998; Gressens, 2000). Environmental events causing injury to the central nervous system (CNS) that have been associated with MCD include: head injury, stroke, and hypoxic-ischemic injury. Insights into the mechanisms of MCD formation during brain development may yield new therapies for seizures associated with MCD and may even provide new strategies for the preventions of MCD during embryogenesis (Kisby et al., 2006; Pettersson et al., 2003).

Oxidative stress is one of the major risk factors for neurodegenerative diseases. Recently, there has been increasing evidence supporting the association between oxidative stress and epilepsy (Kunz, 2002; Waldbaum and Patel, 2010). Mitochondrial

function plays a crucial role in reactive oxygen species (ROS) production. Mitochondrial DNA (mtDNA) variations can cause inefficient oxidative phosphorylation leading to the accumulation of ROS, DNA damage, and increased brain lesion risk. During evolution, several mutations have accumulated in mtDNA, representing specific single nucleotide polymorphisms (SNPs), allowing human populations to be categorized into various mtDNA haplotypes and haplogroups (Petterson et al., 2003; Abu-Amero et al., 2006). The roles of various mtDNA variations in the pathogenesis of MCD are scarce. In this study, it is proposed that mitochondrial haplogroup and mtDNA variations can be risk contributors to MCD. It is possible that the effect of mitochondrial genetic background is influenced by physiologic or environmental conditions, such as the hormonal state of an individual. Whether the same group of mtDNA SNPs or haplogroups affects the risk of greater oxidative damage to mtDNA and of developing epileptic lesion requires further investigation. Since mitochondria may play an important role in modulating oxidative stress, the identification of significant mtDNA SNPs and haplogroups associated with MCD would suggest that mitochondria may be involved in gene-environment interactions that may affect the pathogenic mechanism of disease. Overall, the goal of this study was to investigate the mtDNA background and oxidative damage with an individual's risk of MCD in intractable pediatric epilepsy patients. To achieve this goal brain tissue samples from forty-eight pediatric intractable epilepsy patients (non-neoplastic, n=23 and neoplastic, n=25) who have undergone brain resection surgery at the Miami Children's Hospital, Miami, FL, during 2008-2009 were collected, and as references, eleven normal, non-epileptic, pediatric brain tissues were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland.

Research Objectives

Specific Aims

Aim 1: To compare mitochondrial DNA damage and mitochondrial DNA copy number in MCD and non-MCD pediatric intractable epilepsy patients.

Aim 2: To determine the single nucleotide polymorphism (SNP) in neurodevelopmentally important mitochondrial genes that encode for enzymes known to generate and detoxify reactive oxygen species (ROS) and mitochondrial haplotypes in MCD and non-MCD pediatric intractable epilepsy patients.

Aim 3: To determine the relationship between mitochondrial DNA damage, mitochondrial DNA copy number, mitochondrial haplotypes, and SNP variations in genes that encode for enzymes known to generate and detoxify ROS with the phenotype of MCD and non-MCD in pediatric intractable epilepsy patients.

CHAPTER II

LITERATURE REVIEW

Epilepsy

Epilepsy is a neurological disorder of the central nervous system (CNS) which is characterized by recurrent seizures. Epilepsy results from excessive synchronous firing of neurons in cortical networks (Prasad et al., 1999; Paredes and Baraban, 2002). Seizure disorders are often classified as either symptomatic or idiopathic (cryptogenic) epilepsies. Symptomatic epilepsies are due to an identifiable cause, such as a metabolic disorder, brain trauma, or intracranial tumors; whereas, idiopathic disorders occur in the absence of identifiable causal factors (Mefford et al., 2010).

The overall incidence of epilepsy in North America is approximately 50 per 100,000 persons each year, and the prevalence is 5-10 per 1,000 (Theodore et al., 2006). Thus, more than 3 million people in North America have epilepsy. The incidence of epilepsy is highest for children below five years of age, and the elderly (Donner and Snead, 2006). The majority of epilepsy in North Americans is cryptogenic, that is, there is no identifiable condition or insult. Many childhood epilepsies are refractory to medical medicine (Statstrom et al., 2000).

According to Shinnar and Pellock (2002), epilepsy affects approximately 0.5 to 1% of all children through the age of 16 years. The majority of active epilepsy cases are of childhood onset. About 1.5 million Americans, including as many as 325,000 children between 5 and 14 years of age, have active epilepsy. Every year in the United States, 120

of every 100,000 persons seeks medical attention for a newly recognized seizure, and of these 300,000 patients (40%, 120,000) are children under the age of 18 (Shinnar and Pellock, 2002). The median age of seizure onset is between 5 and 6 years (Shinnar and Pellock, 2002). Overall, epilepsy is the most common treatable serious neurologic disorder in children and young adults. It is also the third most common of all serious neurologic disorders.

Persons afflicted with epilepsy are more likely to report reduced health-related quality of life than controls (Theodore et al., 2006). Individuals with epilepsy tend to have reduced income, less likely to have full-time employment, and suffer from the persistent stigma. According to Smeets et al. (2007), people with epilepsy experience objective restrictions, including those related to driving or working in situations in which they might be liable for injuries. "Mortality is increased in patients with epilepsy, and increased mortality risk in childhood-onset mortality is primarily seen in patients with neurologic abnormalities or intractable epilepsy (Shimmar and Pellock, 2002)." The occurrence of childhood epilepsy appears to have long-term impact on education, employment, marriage, and fertility (Shimmar and Pellock, 2002). Despite the high costs and severe disability, epilepsy may attract somewhat less research funding from public and private sources than other less common chronic neurological disorders (Theodore et al., 2006).

A person with epilepsy may periodically experience epileptic seizures. The most common types of epilepsy include petit mal, psychomotor epilepsy, and grand mal. Petit mal occurs almost exclusively in children. Children experiencing a petit mal seizure lose contact with reality for 5 to 30 seconds but do not lose consciousness or display

convulsions (Van De Graff, 2000). Psychomotor epilepsy involves involuntary lip smacking or hand clapping. In addition, if motor areas in the brain are not stimulated, a person with psychomotor epilepsy may wander aimlessly until the seizure subsides. A serious form of epilepsy, grand mal seizure, is characterized by periodic convulsive seizures that render a person unconscious (Van De Graff, 2000). According to Scher (2003), comparatively less attention has been focused on the pathogenic mechanisms leading to epileptogenesis during the perinatal stage of brain development through one month of postnatal life. Thus, profound brain injury from maternal-placental-fetal-neonatal disease has profound effects on brain development contributing to a spectrum of neurologic disorders including epilepsy, cognitive and behavioral disorders.

Refractory (Intractable) Epilepsy

A significant number of patients with epilepsy will have continued uncontrolled seizures despite the availability of many anti-epileptic medications (Saneto and Wyllie, 2000). According to Beleza (2009), refractory epilepsy is established when there is inadequate seizure control despite using potentially effective AEDs at tolerable levels for 1 to 2 years. Refractory epilepsy patients show increased risk of psychiatric, psychosocial, and medical morbidities. Various studies have suggested that onset of early intractable seizures may be a significant risk factor for impairment of intellectual functioning and adaptive abilities (Saneto and Wyllie, 2000). Resective surgery, based on the removal of the entire epileptogenic area without causing permanent neurological deficit, is one of the treatment forms for refractory epilepsy (Beleza, 2009). In surgical series, focal cortical malformations and low-grade tumors were the most common in infants with intractable epilepsy. According to Saneto and Wyllie (2000), low-grade

tumors included gangliomas, gangliocytomas, dysembryoplastic neuroepithiliomas, and astrocytomas.

The occurrence of epilepsy is highly associated with malformations of cortical development (MCD) which consists of dysplastic neuronal lesions (malformations) (Schwarktzkroin and Walsh, 2000; Hua and Crino, 2003; Hader et al., 2004; Rickert, 2006). The seizure type usually reflects the topology of the malformation. Focal seizures occur with focal or multifocal MCD and secondarily generalized seizures with diffuse or bilateral MCDs (Kuzniecky and Jackson, 2008). Currently, the molecular mechanisms responsible for the pathogenesis of MCD are not known. According to Paredes and Baraban (2002), early-onset epileptic disorders associated with MCD are often resistant to conventional antiepileptic treatments and the regions characterized by disorganized cortex act as seizure foci.

Focal cortical dysplasia (FCD) is commonly found in resected tissue from children afflicted by refractory epilepsy (Hader et al., 2004; Munkata et al., 2007). According to Guerrini et al. (2003), about 40% of children with drug-resistant epilepsy demonstrate cortical malformations. In addition, there is an incidence of about 80% of cortical dysplasia in epileptic children younger than 3 years of age and is the most frequent pathology found in pediatric surgery patients (Cepeda et al., 2006). However, the specific role played by the malformations in epileptogenesis remains unclear. Therefore, neuronal lesion formation and the occurrence of epileptic seizures in children is a great public health concern.

Malformations of Cortical Development

Malformations of cortical development (MCD) are developmental brain lesions. MCD are characterized by abnormal formation of the cerebral cortex (Crino, 2004). MCD appear to represent a profound maldevelopment of the cerebral cortical mantle (Crino et al., 2002). MCD are lesions that may be focal or diffuse. MCD include assorted groups of disorders known as neuronal migration disorders (NMDs) and cortical dysplasia (CD). MCD can affect broad regions of the cerebral cortex such as hemimegalencephaly and classical lissencephaly, or may be restricted to focal areas such as Taylor-type focal cortical dysplasia (FCD) or tuberous sclerosis complex (TSC) (Crino, 2004; Montenegro et al., 2007). In lissencephaly and polymicrogyria the normal 6-layered organization of the cerebral cortex is replaced by a more primitive 4-layered organization. MCD present a broad spectrum of structural changes which appear to result from changes in precursor neuronal or neuronal cells during cortical development at various stages such as: (1) proliferation, (2) migration, (3) differentiation, and (4) apoptosis (Becker et al., 2002).

There is a high clinical association between MCD and epilepsy in infants, children, and adults (Hannan et al., 1999; Schwartzkroin and Walsh, 2000; Pillai et al., 2002; Crino, 2004). However, not all cortical malformations are linked to epilepsy (Schwartzkroin and Walsh, 2000; Crino et al., 2002). It is estimated that MCD accounts for about 20% of all epilepsies (Crino et al., 2002). MCD-related epilepsy may be resistant to anti-epileptic drugs (AEDs) and may require resection. MCD is the most common neuropathologic abnormality encountered in studies from several major pediatric epilepsy surgery centers in which resection was performed to treat infantile

spasms and intractable seizure disorders of childhood (Crino et al., 2002). Advances in brain imaging have demonstrated high frequencies of cortical malformations in childhood epilepsy (Chevassus-au-Louis et al, 1998). In addition, the more severe the MCD, the earlier onset of symptoms and the more severe the epileptic syndrome (Mischel et al., 1995). Thus, MCD are increasingly recognized as causes of mental retardation and epilepsy. Yet, the sequence of events which lead to abnormal cortical development epileptogenesis is not known.

According to Montenegro et al. (2002), there are several reports that indicate prenatal events are likely to be involved in the pathogenesis of MCD. Since brain development commences early in fetal life and continues until adolescence, exposure to environmental chemicals at an early stage may be the leading cause of neurodevelopmental disorders (Allen and Walsh, 1999). In 2006, the National Research Council has concluded that 3% of developmental disabilities are a direct consequence of exposure to environmental neurotoxins such as alcohol, pesticides, heavy metals, and polychlorinated biphenyls (PCBs), and that 25% result from the interaction between environmental factors and genetic susceptibility. In addition, several studies indicate that genetic and environmental factors contribute to causation of MCD and lead to neurodevelopmental delay (Kuzniecky and Barkovich, 2001). Therefore, insights into the mechanisms of MCD formation during brain development may yield new therapies for seizures associated with MCD and may even provide new strategies for the preventions of MCD during embryogenesis (Price and Willshaw, 2000).

According to Schwarzkroin and Walsh (2000), the majority of diffuse types of malformations exhibit normal cell types that are abnormally localized and/or oriented and

are associated with abnormal circuits. In contrast, abnormal cell types such as giant cells and balloon cells that may serve as "pacemakers" of epileptic discharge are characteristics of focal malformations. MCD result from abnormalities occurring in the three different stages of brain development. The three stages of brain development include: (1) neuronal and glial proliferation, (2) migration, and (3) cortical organization (D'Incerti, 2003). The morphology of individual neurons in most MCD is abnormal, which suggests a pervasive disruption of many steps important in neuronal development (Crino et al., 2002). In the occurrence of a seizure, the type of malformation associated with any given type of seizure disorder may vary significantly. For example, infantile spasms (IS) have been associated with lissencephaly, tuberous sclerosis, and cortical dysplasia (CD) (Schwartzkroin and Walsh, 2000). On the other hand, distinct forms of structural malformations may be associated with diverse manifestations of seizure types (Schwartzkroin and Walsh, 2000). Thus, the variability in types of malformations and seizure types reflects the maturational state of the brain, and the brain's tendency to generate either focal or generalized seizures. In addition, the complexity of these relationships is aggravated due to the lack of uniformity in the nomenclature of MCD describing both structural and functional abnormalities.

Origins of MCD

It has been postulated that the stage of development in which the abnormality occurs is linked with the nature of the dysplastic lesion. Developmental neurobiologists have described six distinct periods of cortical development (Schwartzkroin and Walsh, 2000). The six periods of cortical development identified are: (1) pattern formation, (2) cell proliferation, (3) cell fate specification, (4) cell migration, (5) cell differentiation, and

(6) synapse/circuitry formation. During pattern formation the gross divisions of the brain are specified. Neural and glial precursor cells are generated in the ventricular proliferative zone during the cell proliferation period. During cell fate specification the destination and general cell type is determined. Cells migrate from the proliferative zone to travel to their designated destination occurs during cell migration. Cell type manifestation is achieved during cell differentiation. Lastly, specific networks of connectivity, as well as, synaptic pruning and apoptosis are determined during synapse/circuitry formation (Schwartzkroin and Walsh, 2000; Crino et al., 2002; Montenegro et al., 2002).

Complex patterns of gene expression occur in each period of development resulting in specific intracellular and extracellular mechanisms. It is stipulated that a disruption and/or modification of these signal mechanisms result in abnormalities in the cortical structure. For example, increased cell proliferation may produce too many cells and result in an abnormally thickened cortex. Alterations or absence of appropriate migration cues may result in heterotopically placed neuronal populations (Schwartzkroin and Walsh, 2000). Thus, the timing of the abnormality greatly determines the nature of the malformation. Overall, prenatal and/or genetic factors are suspected to play a pathogenic role since neuronal migration to cerebral cortex in humans are believed to occur in the first half of gestation (Palmini et al., 1994).

Etiology of Malformations of Cortical Development

MCD result from abnormalities occurring in the three different stages of brain development. The three stages of brain development include: (1) neuronal and glial proliferation, (2) migration, and (3) cortical organization. The morphology of the

individual neurons found in MCD is abnormal, which suggest a pervasive disruption of many important steps in neuronal development. In the occurrence of a seizure, the type of malformation associated with any given type of seizure disorder may vary significantly. For example, infantile spasms (IS) have been associated with lissencephaly, tuberous sclerosis (TSC), and cortical dysplasia. The variability in the types of malformations and seizure types reflects the maturational state of the brain, and the brain's tendency to generate either focal or generalized seizures. In addition, the complexity of these relationships is aggravated due to the lack of uniformity in the nomenclature of MCD describing both structural and functional abnormalities. However, little is known regarding the molecular and biochemical signals that control proliferation, migration, and organization of the cells involved in either normal or abnormal cerebral cortical development. Yet, several environmental agents such as rubella, lead, methyl mercury, alcohol, retinoids, and thalidomide have been identified to be toxic to the developing central nervous system (CNS) by interfering with specific developing processes (Rodier, 1995).

Development of Cerebral Cortex

The development of the cerebral cortex commences on the seventh week of gestation and continues through the twenty-fourth week (Crino et al., 2002). The cerebral cortex is formed in four stages. During the first stage, mitosis and proliferation of the progenitor cells in the embryonic vascular zone (VZ) and from the ganglionic eminence (GE) occurs. In the second stage of cerebral cortex formation, cells exit mitotic phases of the cell cycle and commit to neural lineage. The third stage is characterized by the dynamic migration of post-mitotic neurons from the VZ and GE. The final stage of

cerebral cortex formation is the establishment of the cortical laminae consisting of six layers (layers I-VI) (Crino et al., 2002). Through the process of cortical lamination, nascent cells migrate from the VZ along the radial glial fibers into the developing cortical plate through an "inside-out gradient." Neurons which are destined to reside in the deeper laminae (layer VI) arrive first in the cortical plate. Thus, the subsequent groups of neurons migrate through each preceding layer and established layer (Crino et al., 2002).

Types of MCD

MCD may be categorized as: (1) cell fate, proliferation, and specification disorders, (2) disorders of neuronal migration, or (3) disorders of unspecified mechanism or developmental context.

Cell fate, Proliferation, and Specification Disorders

Tuberous sclerosis (TS)

MCD that are considered cell fate, proliferation, and specification disorders includes tuberous sclerosis (TS). Tuberous sclerosis (TS) is a disorder which involves multiple organs and occurs in about 1 in 6000 live births (Miloloza et al., 2002; Hengstschlager and Rosner, 2003; Shah and Hunter, 2005). TS is a complex disorder characterized by cortical tubers which are strongly associated with epilepsy (Crino et al., 2002). In TS, neuroradiological features include: (1) subependymal hamartomas, (2) cortical hamartomas, (3) subcortical hamartomas, (4) linear white matter abnormalities, and (5) giant cell subependymal astrocytomas (D'Incerti, 2003). On imaging studies, subependymal hamartomas appear as nodules bulging into the lateral ventricles. According to D'Incerti, the nodules are often calcified and easily recognized on CT. Both cortical and subcortical hamartomas involve the cortex and subcortical white matter,

and there is usually an enlargement of the affected areas. Giant cell astrocytomas are extremely similar to subependymal hamartomas, except that they develop proximally to the foramina of Monro and often cause obstructive hydrocephalus, a disorder often referred to as "water on the brain" (D'Incerti, 2003). Giant cells are a unique cell type that is not seen in other neurological disorders, except severe FCD. Hence, giant cells are a defining facet of TS.

Dysplastic stem cells in the germinal zone give rise to the hamartomas (tubers). In addition, the dysplastic stem cells give origin to dysplastic glial cells, dysplastic neurons, and cells containing both dysplastic glial and neuron characteristics (Soucek et al., 2001; D'Incerti, 2003). The accumulation of disorganized collections of dysplastic cells in the subependymal and cortical regions are a result of the abnormal differentiation of dysplastic cells. Interestingly, the cellular compositions of hamartomas are the same as those found in focal cortical dysplasia with balloon cells (D'Incerti, 2003). Thus, TS involves incomplete or defective migration of cortical neurons.

Tuberous sclerosis is an autosomal dominant genetic disorder (Crino et al., 1996; Ito and Rubin, 1999; Soucek et al., 2001; Wataya-Kaneda et al., 2001; D'Incerti, 2003; Narayanan, 2003, Wong, 2007). TS results from mutations in *TSC1* and *TSC2*, which are two non-homologous genes. Mutations in the *TSC1* and *TSC2* genes have been identified. In *TSC1* the mutation location is 9q34, whereas, the mutation location for *TSC2* is 16p13.3 (Soucek et al., 2001; Wataya-Kaneda et al., 2001; D'Incerti, 2003; Narayanan, 2003, Wong, 2007). The *TSC1* gene encodes for the 130kDa protein known as hamartin (Miyata et al., 2004). According to Crino et al. (2004), hamartin has virtually no homology to known vertebrate genes. The *TSC2* gene encodes for the 200K protein

tuberin (Crino et al., 2004; Miyata et al., 2004). Tuberin's structure differs from that of hamartin's. The mRNA and proteins of both hamartin and tuberin are expressed in normal tissue. Hamartin and tuberin has been found to be expressed in the brain, liver, cardiac muscle, kidney, adrenal cortex, and skin (Crino et al., 2004). Studies have demonstrated that hamartin interacts with the ezrin-radixin-moesin (ERM) family of the actin-binding proteins which may contribute to the cell-cell interactions, cell adhesion, and cell migration (Lamb et al., 2000). According to Paredes and Barbaran (2002), tuberin displays GTPase activating protein (GAP) activity on Rap1 and Rab5, members of the super family of Ras-related proteins. Rap1 is believed to function in the regulation of DNA synthesis and cell cycle transition (Crino et al., 2004). Tuberin promotes entry of the cell cycle into G_o phase and inhibits the G_1/S phase transition (Astridinis et al., 2003). In addition, phosphorylation of tuberin by Akt negatively regulates the inhibition of p70S6K. Hamartin is thought to regulate cell adhesion. According to Becker et al. (2002), loss of heterozygosity (LOH) studies show allelic losses at *TSC1* (9p434) and *TSC2* (16.6p13.3) in lesions of TSC patients and in sporadic tumors of individuals not afflicted with TSC indicates a tumor suppressor gene function Tuberin and hamartin serve to form a tumor suppressor complex that plays a central role in the insulin/PI3K-signalin pathway (Paredes and Barbaran, 2002; Schick et al., 2007a, Wong, 2007). Mutations in *TSC1* and *TSC2*, that promote activation of mTOR, cause benign hamartomas that rarely metastasize (Fisher and White, 2004). Therefore, mutations of hamartin or tuberin in TSC results in the hyperactivation of the downstream of the mTOR pathway and the associated kinase signaling cascades and translational factors which result in increased cell growth and proliferation.

Disorders of Neuronal Migration

Lissencephaly

Lissencephaly is a severe developmental malformation which is highly associated with neurological deficits and epilepsy (Willmore and Ueda, 2002). According to Crino et al. (2004), there are two pathological subtypes of lissencephaly, referred to as (1) type I (classical), and (2) type II (cobblestone). In lissencephaly, there is poor sulcation or the smooth surface of the brain is absent (D'Incerti, 2003). In lissencephaly, there is stunt or arrest of neuronal migration resulting by a disruption of radial glial fibers. As a result of the disruption, there is abnormal lamination of the cortex (usually there is disruption of the neurons in four layers) (D'Incerti, 2003). The terms agyria (no gyri) and pachygyria (broadened gyri) are used to describe the appearance of the surface the brain.

Agyria

Agyria (complete lissencephaly) refers to the absence of sulci on the brain's surface, whereas, pachygyria (few and broadened gyri) is incomplete lissencephaly. Agyria is associated with the deletion of chromosome 17 and it is observed in patients with Miller-Dicker syndrome, which was one of the first MCD genes discovered, *LIS-1* (D'Incerti, 2003; Crino et al., 2004). According to Crino et al. (2004), Miller-Dieker lissencephaly syndrome is an autosomal recessive disorder which is characterized by type I (classic) lissencephaly, craniofacial dysmorphism, profound mental retardation, and epilepsy.

Agyria has been observed in the male offspring of women with affected by band heterotopia. These male offspring present with a mutation in the doublecortin gene (*DCX*) in chromosome Xq 22.3, also known as X-linked lissencephaly (XLIS) (Wilmore

and Ueda, 2002; D'Incerti, 2003; Crino et al., 2004; Wang et al., 2006). XLIS is also characterized by type I lissencephaly. Interestingly, *DCX* gene mutations in females results in the subcortical band heterotopia syndrome. The mutations which occur in the *DCX* gene may result from deletion, nonsense, missense, or splice donor mutations. *DCX* gene encodes a 40kDa protein that is normally expressed during neuronal migration (gestational weeks 12 through 20) (Crino et al., 2004). Thus, *DCX* mutational effects will only be exerted during this neuronal migration. Overall, *LIS-1* and *DCX* mutations account for 70 to 80% of classical lissencephaly syndromes (Crino et al., 2004).

Microlissencephaly

Microlissencephaly is categorized as one of the generalized forms of MCD. Severe microcephaly is the main feature always observed in microlissencephaly (D'Incerti, 2003). It has been postulated that microcephaly may be caused by a disturbance in neuron proliferation. In addition, an abnormal gyral pattern with few gyri and shallow sulci are also observed in microlissencephaly. Yet, based on MRI features and clinical course, the thickness of the cortex is predominantly normal in microlissencephaly.

Microcephaly

Microcephaly has been associated with maternal problems such as: (1) alcoholism, (2) diabetes, and (3) German measles (rubella). Genetic factors may play a role in causing some cases of microcephaly. Newborns affected with microcephaly generally exhibit neurological defects and seizures. In addition, severe impairment in intellectual development and disturbances in motor functions may appear later in life. Waterham et al. (2007) examined a newborn girl with microcephaly and found defects of

the fission of both mitochondria and peroxisomes and a dominant-negative mutation in the dynamin-like protein 1 gene (*DLP1*). *DLP1* has been implicated in the fission of mitochondria and peroxisomes. In addition, over expression of the mutant *DLP1* in control cells reproduced the defects in mitochondria and peroxisome fission (Waterham et al., 2007).

Periventricular nodular heterotopia (PNH)

Periventricular nodular heterotopia (PNH) consists of nodules of gray matter that are located along the ventricles which result from a total failure of the migration of some neurons (Guerrini and Marini, 2006). Many PNH patients present with epileptic seizures. PNH is an X-linked dominant disorder. PNH displays high rates of male hemizygous lethality (Guerrini and Marini, 2006). Mutations in the *FLNA* gene which is located in Xq28 have been identified in PNH. *FLNA* codes for the protein Filamin A. Models of *FLNA* function postulate that *FLNA* acts early in development and acts as a switch required to neurons to become competent for subsequent migration (Guerrini and Marini, 2006). Interestingly, heterozygous *FLNA* females present borderline to normal intelligence and mild to intractable epilepsy.

Disorders of Unspecified Mechanism or Developmental Context

Fukuyama muscular dystrophy syndrome (FCMD)

Fukuyama muscular dystrophy syndrome (FCMD) is an MCD of unspecified mechanism. FCMD is a rare autosomal recessive disorder which exhibit type II lissencephaly (cobblestone lissencephaly) (Crino et al., 2004). FCMD is associated with seizures and debilitating muscular dystrophy. FCMD has been primarily observed in Japan. *FCMD* gene is found in chromosome 9q31 and encodes for the protein fukutin.

FCMD has been primarily observed in Japan, and it is the most common congenital muscular dystrophy in Japan.

Polymicrogyria (PMG)

Polymicrogyria (PMG) is a neuronal disorder in which the cortex exhibits various small microgyri which expose a pattern of a 4-layered lamination (Crino et al., 2004). PMG reflects an irregular process occurring during the late stages of corticogenesis. Montenegro et al.'s (2002), pathologic findings of a necrotic layer in patients with layered PMG supports the traditional theory that these abnormalities are a form of destructive lesion. PMG has been associated with a variety of neurological syndromes. The majority of children with polymicrogyria have some degree of developmental delay or disabilities, feeding difficulties, respiratory problems, mental retardation, and seizures. PMG results from differing causes that may be both genetic and non-genetic in nature, such as: (1) a genetic disorder (inherited in a sporadic manner), (2) viral infection of the baby during the second trimester (i.e. cytomegalovirus infection), (3) insufficient blood supply to the baby's brain during the second trimester (i.e., umbilical cord entanglement), and (4) other causes that have yet to be identified. Yet, the molecular pathogenesis of PMG has not been identified. However, the molecular basis of PMG is beginning to be elucidated with the identification of *GPR56* gene (for bilateral frontoparietal PMG). It is believed that functional studies of the *GPR56* gene product will clarify the causes of PMG, possibly the mechanisms of normal cortical development, and the regional patterning of the cerebral cortex.

Hemimegalencephaly (HME)

Hemimegalencephaly, also known as unilateral megalencephaly, is characterized by a disproportionately enlarged hemisphere (D'Incerti, 2003). The affected hemisphere contains malformations in the cortex and the white matter. Abnormal lamination, cortical heterotopia, and focal pachygyria are cortical abnormalities found in hemimegalencephaly. Calcification of the intracortical may also be present in hemimegalencephaly. The white matter is abnormally increased in this disorder. The opposite hemisphere may be normal. However, subtle malformations and/or even focal dysplasias have been observed radiographically (Crino et al., 2004). Typical clinical manifestations of hemimegalencephaly are hemiparesis, developmental delay, and intractable epilepsy.

Focal Cortical Dysplasias (FCD)

Focal cortical dysplasias (FCD) belong to a group of disorders described as disorders of cortical development, cortical dysgenesis, cortical dysplasias, or neuronal migration disorders (Wang et al., 2006). FCD is closely associated with medically intractable epilepsy (Montenegro et al., 2002; Hua and Crino, 2003). One of the most common neuropathological findings in tissue resected from refractory epileptic pediatric patients is FCD. A surgical series for childhood temporal lobe epilepsy (TLE) revealed FCD in 18% of pediatric patients (Miyata et al., 2004). According to Rickert (2006), studies indicate the pathogenesis for FCD may result from multifactorial influences such as (1) gene mutations, (2) in utero injuries, (3) peri-natal injuries, or (4) post-natal injuries.

According to Hildebrandt et al (2005), FCD present with aberrant architectural organization of the neocortex and the adjacent white matter. Histologically, FCD are defined by disorganized cortical lamination and the presence of a disorganized cortical lamination, heterotopic neurons (HNs), dysplastic neurons (DN), and balloon neurons (BN) that derive from a population of cells or post-mitotic neurons during cortical development (Hua and Crino, 2003). FCDs are classified on either their pathological characteristics or origin of the pathological elements. Histological findings in FCD include architectural abnormalities. The observed abnormalities include columnar disorganization and cortical laminar disorganization. Severe forms of FCD are characterized by the occurrence of abnormal neuronal elements, such as dysmorphic neurons, giant cells, balloon cells, and immature neurons (Wang et al., 2006). Dysmorphic neurons have a distorted axon, cell body, and dendrite morphology which are caused by the accumulation of neurofilaments in the cytoplasm. Giant cells are normal in shape but have an increased cell size, and there appears to be no accumulation of neurofilaments. According to Hua and Crino (2003), FCD's histological features suggest developmental abnormalities affecting select steps during neural proliferation, differentiation or migration. Nevertheless, the precise developmental stage in which FCD are generated is unknown.

The molecular etiology of FCD has been difficult to define since FCD appears to be a sporadic disorder and no family pedigrees have been identified. In addition, since monozygotic twins are discordant for FCD, it has been suggested that FCD may arise through non-inherited and possibly multifactorial mechanisms (Hua and Crino, 2003). However, a study has suggested that polymorphisms in *TSC1* gene is associated with

some FCD cases provides a genetic backdrop for environmental events to alter cortical lamination. Hence, gene mutations or altered gene expression induced by environmental events in several progenitor cells may alter the structural integrity of nascent neurons leading to the heterogeneous and aberrant cell morphologies found in FCD. Over time, researchers have attempted to classify FCD (Rickert, 2006).

Currently, there is no uniform classification system for FCD. Barkowich introduced a classification system which organized several types of MCD according to embryologic and pathophysiologic features. Utilizing Barkovich's classification system, cortical dysplasias associated with FCD may be categorized as (1) mild MCD, (2) FCD type I, and (3) FCD II (Taylor-type) (Rickert, 2006) (Table 1.0).

Taylor-Type FCD

Taylor-Type FCD, also known as FCD_{bc} (FCD-balloon cell type), is a subtype of FCD which has been linked to chronic intractable epilepsy (Becker et al., 2002; Hua and Crino, 2003; Hildebrandt et al., 2005; Wang et al., 2006). In Taylor-Type FCD, histopathological analysis has demonstrated glioneuronal malformations which are greatly similar to cortical tubers in patients with tuberous sclerosis. Taylor-Type FCD lesions display a derangement of the cortical laminar structure, and are composed of dysplastic cytomegalic neurons, and balloons cells. In a surgical series, Taylor-Type FCD was the most common developmental pathology identified (Sisodaya, 2004). The incidence for Tyler-Type FCD is not well known because high-resolution magnetic resonance imaging (MRI) in studies may not always allow its detection.

The molecular etiology of FCD has been difficult to define since FCD appears to be a sporadic disorder and no family pedigrees have been identified. Monozygotic twins

are discordant for FCD. Therefore, it has been suggested that FCD may arise through non-inherited and possibly multifactorial mechanisms. Currently, the exploration for FCD candidate genes is an area of intense research.

Gangliogliomas and Dysembrioplastic neuroepithelial tumors

Gangliogliomas are composed of neoplastic, highly differentiated glial cells, and dysplastic neurons (Schick et al., 2007a). Gangliogliomas are the most frequent tumors found in patients with focal epilepsy (Schick et al., 2007b). Gangliogliomas account for 5% of childhood tumors (Schick et al., 2007a). The histological hallmarks of gangliogliomas are the combination of dysplastic neurons that are combined with neoplastic glial cells. According to Schick et al. (2007a), gangliogliomas' neoplastic nature is provided by the proliferative activity of the glial cells. Several features of gangliogliomas, such as (1) their focal nature, (2) differentiated glioneuronal phenotype, and (3) clinical character, indicate an origin of a developmentally compromised or dysplastic precursor lesion. CD34, a stem cell epitope, is greatly expressed in gangliogliomas (Rickert, 2006; Schick et al., 2007a). Gangliomas are low grade tumors classified as WHO grade I or II (Rickert and Paulus, 2001). Dysembrioplastic neuroepithelial tumors (DNETs), which are comprised of 'floating neurons' and oligodendroglial-like elements, are the second major group of glioneuronal tumors (Table 2.0). DNETs are classified as WHO grade I and considered as a mixed neuroglial tumor and research has suggested a probable developmental defect occurring during the second trimester in utero (Chang et al., 2010; Spalice et al., 2010).

MCD Genes

Information regarding the molecular pathogenesis of MCD has been available only since the past decade. According to Crino et al (2002), MCD may be observed in large chromosomal rearrangements and as the consequence of a single gene mutation. Eight genes have been identified through positional cloning strategies in human pedigrees with inherited types of MCD (Table3.0) (Crino et al, 2002). These genes include: *TSC1, TSC2, LIS-1, DCX,* and *FCMD.* Further research is needed to elucidate other genes involved in MCD. Montenegro et al. (2002) believe that abnormal migration in MCD is mainly genetically determined, either as a familial trait or a de novo mutation, however, prenatal events could act in conjunction with genetic predisposition to determine the final phenotype.

Effects of Environmental Factors and MCD

The developing central nervous system (CNS) is much more vulnerable to injury from toxic agents than the adult (developed) CNS (Rodier, 1995). Environmental events causing injury to the CNS that have been associated with MCD include: hypoxic-ischemic injury, head injury, and stroke. According to Ottman (1997), approximately 25% of prevalent epilepsy is associated with an antecedent central nervous system injury (e.g., head trauma, stroke, or brain infection) and, accordingly, is classified as "symptomatic." Human and animal studies have demonstrated that *in utero* exposure to environmental factors such as (1) teratogenic, (2) physical, and (3) biological factors result in neuronal migrational disorders (Table 4.0). These environmental factors include: (1) infection with cytomegalovirus, (2) infection with toxoplasmosis, (3) ethanol, (4) cocaine, and (5) ionizing radiation (Chevassus-au-Louis et al., 1999; Gressens, 2000).

In addition, the effect of environmental agent on the developing brain differs depending on the stage in which the insult is delivered (Rodier, 1995).

Teratogenic Factors

Alcohol and cocaine interfere with neurogenesis and neuronal migration which results in microcephaly and disorganized cortical cytoarchitecture. In addition, administration of cocaine to pregnant mice and monkeys induce abnormal addressing of neurons in the neocortical plate (Fantel, et al, 1997; Gressens, 2000). Animal models induce cortical dysplasia though the exposure of (1) freeze lesion, (2) irradiation, and (3) methlylazoxymethanol (MAM) (Bernadete and Kriegstein, 2002). The MCD model that most realistically replicates the morphology of human cortical dysplasia is based on the prenatal exposure to MAM (Chevassus-au-Louis,1998; Gressens, 2000; Choi, 2005; Harrington et al., 2007). MAM is an anti-proliferative, cytotoxic, and DNA alkylating agent that induces cerebral heterotopias that are very similar to those observed in human cortical dysplasia. MAM alters migration and differentiation of neurons leading to heterotopia. Injection of MAM into a pregnant rats at day 14 or 15 exposes the fetuse(s) disrupts cell proliferation at the time of neocortical and hippocampal neuron formation. Prenatal MAM exposure results in cortical dysplasia, microcephaly, periventricular heterotopia, and hippocampal heterotopia (Watanabe, et al., 1998). Prenatal exposure to thalidomide in the rat model results in the inhibition of angiogenesis and significant morphological alterations in cortical and hipocampal regions (Hallene et al., 2006). Thalidomide exposure resulted in abnormal neuronal development that was associated with vascular malformations and a leaky blood-brain barrier. In addition, neuronal hyperexcitability was found in these abnormal regions (Hallene et al., 2006). However,

the mechanisms by which all of these teratogenic environmental factors disturb neuronal migration are not known.

Physical Factors

In animal studies, freeze lesions are produced by touching a freezing probe to the skull surface of the newborn rats. Loss of normal cortical layering beneath the site of contact occurs. As a result of microgyrus develops with the formation of a four-layered cortex instead of a normal neocortex (Schwartzkroin and Walsh, 2000). In addition, the number of cells reduces in the dysplastic region in freeze lesion. Ionizing radiation is an environmental agent with the property of killing neurons (Rodier, 1995). Irradiation of fetal rats at day 12 through 19 produces cortical abnormalities which range from subtle cortical thinning to a dramatic cell loss and dysplasia. Irradiation exposure interferes with DNA replication in proliferating cells (Schwartzkroin and Walsh, 2000). Irradiation treatment effects range from subtle cortical thinning, loss of cell numbers, to severe dysplasia. Lastly, other prenatal traumas such as hypoxia can damage the radial glial fibers and may give rise to migration abnormalities resulting in heterotopic cell clusters and/or thinned cortex and may be accompanied by abnormal cortical lamination.

Studies have shown that during and after hypoxic injury vascular endothelial growth factors (VEGF) is upregulated. There are four VEGF-like families: (1) placental growth factor (PIGF), (2) VEGFB, (3) VEGFC, and (4) VEGFD. VEGF induces angiogenesis, vascular permeability, and inflammation (Croll et al., 2004; Troost et al, 2008). VEGF has been implicated in the breakdown of the blood-brain-barrier after hypoxic injury. After pilocarpine-induced status epilepticus (SE) there is an increase of VEGF in neurons and glia. In addition, to the breakdown of the blood-brain-barrier,

hypoxic injury also results in endothelial apoptosis, upregulation of adhesion molecules, and angiogenesis. VEGF also activates glia and could impact seizure. VEGF also induces inflammation that is characterized by monocytic infiltrate. VEGF is upregulated by IL-1 and TNF-α, coincidentally, these cytokines are upregulated after seizures. Hence, suggesting that IL-1 and TNF-α increase the potential for seizures (Croll et al., 2004). Interestingly, VEGF also presents neuroprotective and could protect vulnerable cells from damage associated with seizures. VEGF protects cultured hippocampal neurons against glutamate excitotoxicity and seizure-induce injury (Croll et al., 2004). VEGF exerts its neuroprotective actions by activating the Akt survival pathway. Thus, in response to hypoxic insult VEGF's actions are both damaging and neuroprotective.

According to Troost et al. (2008), members of the VEGF family are key signaling proteins in angiogenesis induction and regulation during development and pathological conditions. Signals mediated through the VEGF family proteins and their receptors have demonstrated direct effects on neurons and glial cells. Troost et al (2008) investigated the expression of VEGFA, VEGFB, and their receptors (VEGFR-1 and VEGF-2) in FCD type IIB with intractable epilepsy. Their study findings demonstrated a high expression of VEGFA, VEGFB, and VEGF receptor in the dysplastic neurons. The high expression of both VEGFA and VEFG with their receptors in the FCD specimens suggests autocrine/paracrine effects on dysplastic neurons. These effects could play a role in FCD development by the death of abnormal neurons (Troost et al., 2008).

Biological Factors

Maternal disease may contribute to uteroplacental insufficiency syndromes that may alter brain development after injury throughout the ante partum period or during

stresses of labor and delivery. For example, pathophysiologic mechanisms that are involved in asphyxia and inflammation may be expressed by placental function and structures. Placental vasculopathies or pathological lesions which may adversely affect fetal organ structure and function may result from the injury to the villus, stromal, or vascular components within maternal or fetal surfaces of the placenta (Scher, 2003). In addition, fetal brain infections may result in brain malformation. Prenatal infection with toxoplasmosis has resulted in brain malformation, mental retardation, and seizures (Gressens, 2000). Other fetal brain infections that have been found in brain malformations are rubella, cytomegalovirus, and herpes simplex.

Gene-environment Interactions and MCD

Since MCD may result from an abnormality during prenatal brain development, the gene-environment interactions must be considered. The developing organism is a biological system that is maturing over specific time intervals during which adverse conditions result in environmental stress. The time intervals in which environmental stress may occur are embryonic, fetal, and perinatal periods. These adverse conditions include exposure to environmental factors (teratogenic, physical, or biological) and environmental events (i.e. hypoxic-ischemic injury, head injury, and stroke). The stage in brain development in which these events occurs influence the expression of a disease process resulting in brain injury that may expressed as neonatal seizures and epileptic disorders (Kisby et al., 2006). Thus, post-mitotic alterations in structure and functions may result epigenetic effects combined with inherited traits.

As previously mentioned, MCD are traditionally classified by the prenatal stages of brain development. Yet, there is genetic variability of expression are observed through

the variable phenotype expressions of the same disorders as described for tuberous sclerosis. After environmental stress pleomorphisms can define a disease with differing patterns of malformation. Differing brain lesions reveal the stage of neural developmental process and the susceptibility of the specific brain regions in response to injury. Gene expression alterations for specific molecular processes on the cells, such as neurotransmitters and synapses will later assist in the occurrence of neonatal seizures and later as epilepsy during childhood.

Epigenetics

Epigenetics refers to the heritable traits (over rounds of cell division and sometimes transgenerationally) that do not involve changes to the underlying DNA sequence. It is important to recognize that a large percentage of early-onset seizure disorders, mainly associated with cortical encephalopathy, are generated by epigenetic influences, such as trauma to the immature brain which occur during pregnancy or during the birth process (Schwartzkroin and Walsh, 2000). The effects of epigenetics on brain function and structure have been demonstrated in animal models of asphyxia. After an acute asphyxial insult there are changes in global gene expression within the immature brain. In presynaptic and postsynaptic activities epigenetic effects result in either up-regulation or down-regulation of gene activities. Hence, epigenetic alterations in gene-expression the asphyxial model resulted in global alterations in synaptic brain activity (Schwartzkroin and Walsh, 2000). Hence, in MCD gene-environment interactions may grade degrees of risk in the context of genetic pleomorphism and environmental factors. Gene profiles may predispose specific mother-fetal pairs to the harmful effects of environment factors such as trauma, infection, and asphyxia.

MCD Epileptogenesis

Many studies addressing environmental factors that result in MCD try to identify the mechanisms that result in the epileptogenesis in MCD. Abnormal electrical discharge in the brain results in epileptic activity (Paredes and Baraban, 2002). Currently, there are several hypotheses regarding the mechanisms of epileptogenesis in MCD, primarily in FCD. These hypotheses may be classified whether they stipulate that the seizure starts within the lesion or the perilesional (surrounding) regions. According to Paredes and Baraban (2002), aberrant electrical activity in cortical malformations could result from synaptic changes that may occur on the postsynaptic cell itself, such as receptor alterations. Various studies have demonstrated abnormalities in postsynaptic neurotransmitter receptor subtypes in resected dysplasia tissue.

Neuronal Excitotoxicity

Neurons are specialized brain cells for the rapid transmission of information over long distances. Neurons receive information at their dendrites and integrate the information in the cell body (Raven and Johnson, 1996). Information is transmitted in neurons in the form of electrical impulses that are sent out from the cell body along the axon. Excitotoxicity contributes to neuronal degeneration and may play a role in epilepsy (Arundine and Tymianski, 2003). Excitotoxicity is the pathological process in which neurons are damaged and killed by the over- activation of receptors by excitotoxins such as neurotransmitters (Aarts and Tymianski, 2003). Neurotransmitters which act as excitotoxins are N-methyl-D-aspartate (NMDA), kainic acid, and glutamate. High levels of calcium ions (Ca++) enter the cell due to the excitotoxin's actions. The high influx of Ca++ activates several enzymes which damage the cell's structures and DNA. The

activated enzymes include phospholipases, endonucleases, and proteases. Neuronal damage and death are associated with lesions which result in epileptic seizure. In addition, among the mechanisms that govern neuronal migration, the neurotransmitters GABA and glutamate deserve particular attention because neurotransmitters and receptors are expressed early in the developing brain, neurotransmitters may act as paracrine signaling molecules in the immature brain, and neurotransmitters regulate intracellular calcium required for many cellular functions, including cytoskeletal dynamic changes (Manent and Represa, 2007).

Transmission of Nerve Impulses: Neuron Communication

Neurons are known as voltage-gated ion channels because membrane potential changes in response to neurotransmitter stimulation (Raven and Johnson, 1996). The diffusion of Na+ and K+ can result in an impulse (action potential). In neurons, the Na+ and K+ channels differ from those in most cells because the channels have gates that open and close when the membrane potential is altered. Action potentials result when depolarization reaches a threshold. Action potentials follow an all-or-none law (Raven and Johnson, 1996). Action potentials pass down the axon and eventually reach the end of the axon and its branches. The arrival of the action potential to the end of the axon causes the membrane to depolarize and results in the release of neurotransmitters from the synaptic cleft. The neurotransmitter, glutamate, binds to the receptor proteins of the postsynaptic cell. The binding of glutamate to the receptor open chemically gated ion channels found on the postsynaptic membrane. In contrast to the voltage-gated channels in the axon membrane, these postsynaptic channels are not opened by membrane

depolarization (Raven and Johnson, 1996). Chemical junctions are an advantage because different neurotransmitters can elicit postsynaptic cell response.

Glutamate Receptors

Glutamate receptors play a vital role in the mediation of excitatory synaptic transmission. Glutamate exerts its signaling role by acting on glutamate receptors (Danbolt, 2001). Activation of glutamate receptors is responsible for synaptic transmission, and several forms of synaptic plasticity. Glutamate receptors are categorized as either metabotropic (G-protein coupled) receptors or ionotropic (ion channel gated) receptors. A review of the literature available on glutamate receptors revealed more information regarding ionotropic glutamate receptors actions in glutamate mediated excitotoxicity than metabotropic glutamate receptors.

Mitochondria can modulate neuronal excitability and synaptic transmission. In epilepsy, the energy consumption and the Ca++ load of neuronal cells increases during epileptiform activity (Kunz et al., 1999). The opening of the Ca++ channels in the mitochondria causes oxidative stress through the release of free radicals. The free radicals cause DNA damage which lead to cell damage and/or apoptosis. In addition, mitochondria lack many of the mechanisms to repair DNA damage caused by radical oxidation. Therefore, the mitochondria can be damaged at the DNA level. Neuronal damage and death leads to lesions which are associated with epileptic seizures. According to Waldbaum and Patel (2010), mitochondrial oxidative stress and dysfunction are emerging as key factors that not only result from seizures, but may also contribute to epileptogenesis.

Mitochondria and Oxidative Stress

Mitochondria

Mitochondrial oxidative stress and dysfunction are contributing factors to various neurological disorders (Waldbaum and Patel, 2010). Mitochondria are tubular organelles 1 to 3 μm long that are found in all types of eukaryotic cells. Mitochondria are double membrane organelles that change their size and position in their dynamic movement. Mitochondrial morphology varies in response to environmental and cellular differentiation. They form connected and filamentous network structures in fibroblast and are arranged along the myofibrils in skeletal muscle, and are coiled around the flagella in sperm (Tagauchi et al., 2007). The outer membrane in mitochondrion is smooth, whereas the inner membrane is folded into numerous layers referred to as cristae (Raven and Johnson, 1996, p.102). There are two compartments to the cristae: (1) a matrix (lye inside the inner membrane) and (2) the outer compartment /intermembrane space (lye between the two mitochondrial membranes). Proteins that carry out oxidative metabolism (the oxygen-requiring process by which energy in macromolecules are stored in adenosine triphosphate [ATP]) are found on or within the surfaces of the inner membrane. Biochemical evidence suggests the majority of cerebral ATP consumption is used for operation of the electrogenic activity of neurons (Chen et al., 2010). Mitochondria also functions in heme, lipid, amino acid biosynthesis, and fatty acid oxidation among other functions (Sugimoto, 2008). Hence, mitochondria are essential for energy production in the cell and are often referred to as the “power plant” for eukaryotic cells.

Mitochondrial Production of Reactive Oxygen Species

ATP is the energy currency utilized by cells. The ATP molecule transfers the energy captured during respiration to the many sites in the cell where it is used (Raven and Johnson, 2004, pp.187). ATP is composed of a sugar (ribose) which is bound to an organic base (adenine) and a chain of three phosphate groups (each phosphate group is negatively charged). Energy is released when the phosphate bonds in ATP are hydrolyzed.

Cells produce ATP in two different ways: (1) substrate-level phosphorylation, and (2) electron transport chain. In substrate-level phosphorylation, ATP is formed by transferring a phosphate group to an adenosine diphosphate (ADP) from a phosphate-bearing intermediate (Johnson and Raven, 1996). For example, during glycolysis the chemical bonds of glucose are shifted around in reactions that provide the energy needed to form ATP. Even though this process is inefficient, many cells utilize substrate-level phosphorylation to derive their ATP. In the second process, electrons are harvested and transferred in the electron transport chain. Most organisms combine these two processes in the oxidative phosphorylation.

Oxidative phosphorylation is accomplished through a complex series of enzyme-catalyzed reactions that may be broken down into four stages. For example, the first stage of extracting energy from glucose is a 10-reaction biochemical reaction pathway known as glycolysis. During glycolysis, the enzymes needed to catalyze the glycol tic reactions are found in the cytoplasm of the cell. During glycolysis two ATP molecules are utilized to prepare glucose, and four ATP molecules are created through substrate level phosphorylation. Thus, glycolysis only yields two ATP molecules per glucose

molecule that is catabolized (Raven and Johnson, 1996). In addition, once the glycolysis process is complete two pyruvate molecules are formed. In the second stage, the pyruvate is converted into carbon dioxide and acetyl-CoA. In the third stage, acetyl-CoA is introduced into a cycle of nine reactions known as the Krebs cycle (citric acid cycle), which generate two ATP molecules. In the final stage, the electrons carried by NADH are used to drive the synthesis of ATP by the electron transport chain. In addition, glycolysis can occur in the absence of oxygen. However, the harvesting of electrons for the electron transport chain can't take place indefinitely in anaerobic conditions because oxygen serves as the final electron acceptor of the electrons harvested from glucose. Therefore, without oxygen cells are restricted to substrate-level phosphorylation to obtain ATP, and some organisms respire by utilizing different electron acceptors. Overall, pyruvate oxidation, Krebs cycle, and ATP production takes place in the mitochondria of all eukaryotic cells.

Mitochondrial metabolism also generates the majority of the reactive oxygen species (ROS) production in cells (St-Pierre et al., 2006). ROS results when unpaired electrons escape the electron transport chain. The generation of ROS in normal cells, including neurons, is under tight homeostatic control. Antioxidants such as glutathione, vitamin E, carotenoids, and ascorbic acid help to detoxify ROS by reacting with most oxidants (Klein and Ackerman, 2003). ROS react with molecular oxygen to generate superoxide. It is estimated that under normal physiological conditions up to 1% of the mitochondrial electron flow leads to the formation of superoxide (O_2-) and interferences in electron transport increases O_2-. However, oxidative stress in not limited to mitochondrial metabolic processes because ROS may be produced by environmental

events such as: (1) ionizing radiation exposure, (2) cytotoxic chemical exposure, (3) drug exposure, or (4) by professional phagocytosis resulting from the defense against invading pathogens (Langley and Ratan, 2004).

Superoxide is the primary ROS created by mitochondria. Within the cell O_2- is rapidly converted to hydrogen peroxide (H_2O_2) by superoxide dismutases, SOD1, SOD2, and SOD3. Through a Fenton reaction H_2O_2 reacts with reduced transition metals to produce the high reactive hydroxyl radical (-OH). O_2- also reacts with nitric oxide (NO) to create cytotoxic peroxynitrite anions (ONOO-). Peroxynitrite reacts with carbon dioxide which results in protein damage through the formation of nitrotyrosine and lipid oxidation (Klein and Ackerman, 2003). Hence, ROS can also react with nitrous oxide (NO) to generate reactive nitrogen species (RNS). Superoxide reacts with DNA, proteins, and lipids. O_2- selectively attacks guanine. The most commonly produced base lesion, and the most often measured as an index of oxidative DNA damage is 8-hydroxyguanosine (8-OHdG) (Wiseman and Halliwell, 1995). O_2- plays an important role in several physiological and pathophysiological conditions such as ischemia-reperfusion injury, neurodegenerative disease, and aging (St-Pierre et al., 2006).

According to Finkel and Gutkind (2003), there is a growing consensus that oxidative stress plays an integral role in both aging, and the pathophysiology of most neurodegenerative diseases. It is apparent that oxidative stress induced injury in the nervous system results from the overproduction of oxidants and/or the dysfunction of endogenous oxidant defenses (Langley and Ratan, 2004; Harrison et al., 2005). In addition, ROS can also cause damage to the mitochondrial genome (Fishel et al., 2007).

Therefore, the maintenance of low ROS levels is crucial to normal cell functions, and prolonged increase in mitochondrial activity inherently risk increasing ROS levels.

Mitochondrial DNA damage during epileptogenesis

Acute consequences of status epilepticus (SE) are oxidative stress and mitochondrial dysfunction (Jarret et al., 2008). Currently, the role of mitochondrial oxidative stress and genomic instability during epileptogenesis is not known. Jarret et al. (2008) used the kainite rat model of temporal lobe epilepsy (TLE) to investigate mitochondrial DNA (mtDNA) damage and changes in the mitochondrial base excision repair pathway (mtBER) in the hippocampus for 3 months after SE. Results of the study demonstrated a time-dependent increase in the frequency of mtDNA lesions. The increase in mtDNA lesions was accompanied by an increase in mitochondrial H_2O_2 production and a decrease of mtDNA repair capability (Jarret et al., 2008). In addition, there was an elevated expression mRNA and protein of the mtBER protein 8-oxoguanine glycosylase (Ogg1) and DNA polymerase gamma (Pol gamma). Hence, the increase of oxidative mtDNA damage, mitochondrial H_2O_2 production and alterations in the mtBER pathway present evidence for mitochondrial oxidative stress in epilepsy. Thus, suggesting that mitochondrial injury may be a contributing factor in epileptogenesis.

ROS, Mitochondria and the Neuron Cell Cycle

Examination of the current literature on the effect of oxidative stress on the cell cycle reveals increases in ROS-induced DNA damage are correlated with cell cycle arrest. ROS have been implicated in cell signaling, specifically through mitogens (Klein and Ackerman, 2003). The discovery that oxidative stress can trigger a program of cell death in neurons with features of apoptosis was significant (Langley and Ratan, 2004).

During embryonic development neuronal precursors produce larger numbers of neurons than needed, and this excess in neurons are later eliminated by apoptosis during a restricted embryonic period (Langley and Ratan, 2004; Liu et al., 2004). Even though neuronal apoptosis plays a crucial role in the development of the nervous system, it is also an underlying element in neurodegenerative diseases (Becker and Bonni, 2006). According to Langley and Ratan (2004), there is a growing body of evidence indicating that deregulation of the cell cycle can either (1) trigger apoptosis, or (2) increase sensitivity to apoptotic inducers. In 1994, the first indication that mitochondria play an active role apoptosis occurred. The observation that mitochondria were needed for nuclear apoptotic changes, such as: (1) chromatin condensation, and (2) nuclear fragmentation, in the extracts of *Xenopus* eggs (Crompton, 2000). These observations lead to insights of mitochondrial role in apoptosis. According to Crompton (2001), apoptosis is executed by caspases, proteolytic enzymes that are expressed constitutively as inactive proenzymes, and that are activated by cleavage of the N-terminal prodomain. In addition, it is well recognized that ROS are formed during the reperfusion of ischemic tissue and may result in brain malformations and uteroplacental ischemia during the fetal stages of gestation (Fantel et al., 1998).

The central nervous system (CNS) is vulnerable to oxidative stress. The major reasons being that the CNS (1) posses a low level of antioxidant enzymes, such as catalase and gluthathione peroxidase, (2) have a high content of easily oxidized substrates such as membrane polyunsaturated lipids, and (3) an inherently high flux of ROS generated during neurochemical reactions such as dopamine oxidation and energy metabolism (Langley and Ratan, 2004). The increased and unobstructed ROS can lead to

death via several pathways including necrosis or apoptosis. The most common oxidative product of DNA is 8-hydroxy-2'-deoxyguanosine (8-OHdG), a major DNA lesion resulting from free radical attack that has been demonstrated to alter the base-pairing properties (GC -> TA transversions) of guanine in *in vitro* assays (Kasai, 1997). According to Lin et al (2002), under enhanced oxidative stress, the amount of 8-OHdG in mtDNA has been found to increase although base excision repair (BER) system of oxoguanine glycosylase (hOGG) and DNA ligase are present in the mitochondria. Human oxoguanine glycosylase 1 (hOGG1) recognizes 8-OHdG and catalyze, through cleavage of the N-glycosyl bond between the sugar and the base to generate an apurinic/apyrimidinic (AP) site, resulting in the removal of phosphodiester bond in modified DNA (Wei and Lee, 2002). Inactivation of hOGG1 may result in the accumulation of point mutations and deletions in mtDNA (Higushi, 2007). The amount of 8-OHdG is elevated in both neuronal nuclear and mitochondrial DNA in disease regions of patients with neurodegenerative disorders and has been correlated with increase incidence of cancer and cell cycle abnormalities (Klein and Ackerman, 2003). According to Wiseman and Halliwell (1995), oxidative DNA base damage, measured as 8-OHdG, has been detected in mitochondrial DNA at steady-state levels several-fold higher than in nuclear DNA. This apparent increased net oxidative damage in mitochondrial DNA compared with nuclear DNA may be due the proximity of mitochondrial DNA generated during electron transport, lack of histone proteins to protect DNA against attack, or inefficient repairs, resulting in base damage accumulates in higher levels in mitochondrial DNA. Two markers of oxidative DNA damage are thymine glycol (TG) and 8-OHdG. According to Waldbaum and Patel (2010), TG is an

adduct that can lead to cell death by blocking polymerase action. It has been shown that 8-OHdG is one of the important oxidative lesions related to mitochondrial dysfunction and aging (Lin et al., 2002). Mutations that impair the mtDNA BER pathway have been linked with chronic epilepsy (Jarrett et al., 2008).

Oxidative stress can cause other mutational events such as: (1) strand breaks, (2) discontinuous loss of heterozygosity, (3) large deletions, (4) protein/DNA-cross-links, and (5) modification of base pairs (Waldbaum and Patel, 2010). Hence, mutations may result if the oxidation of DNA surpasses the cell's DNA-repair capacity leads to loss of genome stability. However, cumulative damage in neurons is contingent on other factors of susceptibility, such as: (1) exposure to environmental genotoxins, (2) polymorphisms in genes that are involved in either cellular functions or in metabolism of toxins (Klein and Ackerman, 2003). Animal models of hypoxia-ischemia and traumatic brain injury (TBI) has demonstrated developmental differences in apoptotic neuronal death. According to Robertson (2004), the mechanisms responsible for these differences are unknown, but it is stipulated that they are likely multifaceted and related to mitochondrial response to injury. Mitochondrial involvement and oxidative stress may be contributing factors in neurodegenerative disorders (Sas et al., 2007). Currently, there is ongoing research in the field of neurodegeneration to understand how genetic and environmental factors plot to tip the balance of oxidant and antioxidants in favor or oxidants in the CNS (Langley and Ratan, 2004).

Mitochondrial DNA and Point Mutations

Mitochondria contain their own DNA as a circular duplex. mtDNA is inherited maternally with a vertical non-Mendelian pattern (Solano et al., 2001). Interestingly,

mtDNA located in the cytoplasm from the ovum is transmitted to the zygote and sperm rarely contribute mtDNA to the zygote. This results because there is a high number of mtDNA in the ovum, about 100,000 and 200,000 copies compared to the few hundred in spermatozoids (Solano et al., 2001). In addition, the spermatozoid mitochondria that can enter the fertilized egg are eliminated through an active process. Hence, mothers and all of their offspring share the same mtDNA. mtDNA is randomly distributed to daughter cells in a process known as replicative segregation; and only the daughters will pass the mitochondrial genome to all of the members of the next generation. Heteroplasmy occurs if there is a mixture of mutant and normal mtDNA. According to Sherman (1997), the proportion of mutant to normal mtDNA can change after one cell division. Any two offsprings are likely to receive differing proportions of mutant mtDNA from a heteroplasmic mother. Therefore, the mtDNA resulting in the offspring can be in one of three states: (1) a mixture of normal and mutant mtDNA (heteroplasmy), (2) purely normal, or (3) purely mutant mtDNA (homoplasmy). Hence, this trend may potentially cause the variable phenotypes observed in the offspring. Mitochondrial genetics differ from Mendelian genetics. Some of the peculiarities of mitochondrial genetics are uniparental inheritance, cellular polyploidy, and a deviation from the standard genetic code. These features dictate the functional consequences of pathogenic mtDNA mutations (Tuppen et al., 2010).

mtDNA produces some of the proteins needed for mitochondrial oxidative metabolism (Raven and Johnson, 1996; Wang et al., 2006). Each mitochondrion contains multiple copies of a 16,569 base-pair circular DNA duplex. mtDNA encodes 13 subunits of enzyme complexes which play a role in energy production, including seven subunits

(ND1, ND2, ND3, DN4L, ND5L, and ND6) of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I), one subunit (cytochrome b) of ubiquinol-cytochorme c oxidoreductase (complex III), three subunits (COI, COII, and COIII) of cytrochrome c oxidase (complex IV), and two subunits (ATPases 6 and 8) of complex V with 22 tRNAs and two rRNA (12S and 16S) subunits (Tsutsui et al. 2009). However, the majority of the genes that produce the proteins (enzymes) utilized in oxidative metabolism and divisions are located in the nucleus (Sherman, 1997; Jeng et al., 2008). Mitochondrial replication would be impossible without nuclear participation, and mitochondria cannot be grown in a cell-free culture (Johnson and Raven, 1996). Damaged mitochondrion can replicate because the nuclear DNA (nDNA) encodes the enzymes needed for mitochondrial replication. Increased abundance of defective mtDNA that encodes for respiratory enzymes may lead to impaired electron transport, result in enhanced production of ROS, further oxidative damage, and damage to the mitochondria (De la Monte et al., 2000). mtDNA mutations can be categorized as those that impair mitochondrial protein synthesis and those that affect any of the 13 respiratory chain subunits encoded by the mitochondrial genome (DiMauro, 2007). For example, deficiency of primary coenzyme Q10 (CoQ10) is included as a respiratory chain disorder due to its pivotal role as an electron carrier from complex I and II to complex III. CoQ10 deficiency encompasses fiver major phenotypes such as encephalomyopathy, severe infantile multisystemic disease, Leigh syndrome, isolated myopathy, and cerebellar ataxia (DiMauro, 2007). Advances in mitochondria research have revealed that mutations in the mtDNA lead to a number of genetic disorders.

According to Sherman (1997), the full range of diseases that have a mitochondrial component are unknown, yet, clearly involve disorders such as: acute leukemia, Alzheimer's disease (AD), cardiomyopathy (CM), chronic progressive external ophthalmoplegia, colorectal cancer, deafness, fatal infantile cardiomyopathy (FICP), gastroesopageal reflux (GER), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), Leber Hereditary Optic Neuropathy (LHON), Parkinson's disease (PD), maternally inherited hypertropic cardiomyopathy (MHCM), multiple sclerosis (MS), non-insulin dependent diabetes mellitus (NIDDM), Chronic intestinal pseudo-obstruction (CIPO), non-syndromic hearing loss, progressive encephalopathy (PEM), sensorineural hearing loss (SNHL), strokes, and sudden infant death syndrome (SIDS) (Table 5.0) (Filiano et al., 2002; Bai et al., 2007; Tzen et al., 2007). A list of all of the known disorders associated with mtDNA variations is available at MITOMAP: Human Mitochondrial Genome Database. Diminished mtDNA repair mechanisms and mitochondrial genomic instability have been implicated as important factors in several neurodegenerative diseases (Jarrett et al., 2008). MEERF and MELAS are the most prominent examples of the occurrence of epileptic seizures caused by mitochondrial dysfunction (Kunz, 2002; Chen et al., 2010). The mechanisms of mitochondrial dysfunction during epileptogenesis are unclear.

Mitochondrial DNA Copy Number Variations

According to Liu et al. (2006), the number of mitochondria per cell is maintained within a constant range in response to the energy need of the cell to maintain normal physiologic functions. mtDNA mutations often result in respiratory chain defects

resulting in decreased ATP production, enhanced ROS and free radical production in mitochondria. Mitochondrial dysfunction or cell apoptosis result upon the decline in the copy number of mtDNA or the accumulation oxidative damage and mtDNA mutations reaches a critical point, thus, resulting in defective energy metabolism of the target tissues (Liu et al., 2006). Alteration of mtDNA copy number in affected copy numbers has been suggested play a role in the pathogenesis and progression of mitochondrial diseases (Liu et al., 2006; Park et al., 2009). Studies have documented the presence of inheritable copy number variations (CNVs) in the human genome, and copy number aberrations (CNAs), which are acquired somatic alterations, have been observed in tumor tissues (Sun et al., 2009; Zhang et al., 2010). For example, in nasal polyp tissues, an increase of mtDNA is the result of a compensatory mechanism thought to be triggered by endogenous and exogenous oxidative stress elicited by mtDNA mutations (Park et al., 2009). Additionally, in the skeletal muscle of patients with mitochondrial encephalomyopathies such as MERRF, MELAS, and CPEO demonstrated proliferation of abnormal mitochondria (Liu et al., 2006). Research has suggested that an increase in mtDNA content in affected tissues result from a compensatory mechanisms triggered by elevated endogenous and exogenous stress that may be elicited by mtDNA mutations. Diseases caused by an excess mtDNA proliferation are less common (Montier et al., 2009).

In contrast, mtDNA copy number was found to decrease in tissues from patients with neonatal giant-cell hepatitis, mtDNA depletion syndrome, mitochondrial neurogastrointestinal enchephalomyopathy, renal cell carcinoma, liver disease, biliar atresia, type 2 diabetes, cardiomyopathy, and breast cancer (Liu et al., 2006; Montier et

al., 2009). Interestingly, even though there are a limited number of human studies which directly evaluate mitochondrial function after a traumatic brain injury (TBI), they have generally supported the findings seen in animal models of TBI. Mitochondria isolated from the brain of human victims of TBI have demonstrated impaired rates of respiration and ATP synthesis (Robertson, 2004). In addition, depletion of mtDNA is one of the hallmarks of mtDNA dysfunction (Lewit et al., 2007). These findings indicate that either and increase or decrease in mtDNA copy number may play a role in the pathogenesis of mitochondrial disorders.

Mitochondrial DNA Variations, Haplotypes, and Haplogroups

Point mutations, nucleic acid modifications, and large-scale deletions are the most prevalent forms of mtDNA mutations. All of which play a role in mitochondrial dysfunction and apoptosis. mtDNA damage, as a result of environmental insults and/or enhanced by genetic predisposition, is attracting attention as the origin of mitochondrial dysfunction. During evolution, several mutations have accumulated in mitochondrial DNA (mtDNA), representing specific single nucleotide polymorphisms (SNPs), allowing human populations to be categorized into various mtDNA haplotypes (combination of several SNPs on single chromosomes) and haplogroups (Petterson et al, 2003; Pakendorf and Stoneking, 2005; Abu-Amero et al, 2006) (Table 6.0).

According to Pakendorf and Stoneking (2005), haplogroups represent related groups of sequences that are defined by shared mutations and tend to show regional specificity. Analysis of population-specific mtDNA polymorphisms has allowed the reconstruction of human pre-history, and analysis of maternally inherited diseases has demonstrated that some mtDNA diseases show a strong continental bias (Wallace et al.,

1999). mtDNA polymorphism have sequentially accumulated as women migrated west of Africa and into the other continents. According to Wallace et al. (1999), selection and drift may have influence the rapid shift in the mtDNA lineages that are observed between continents. Thus, the accumulated mtDNA mutations and variations that are observed in high frequency are associated with specific mtDNA haplotypes and haplogroups.

Analysis of human mtDNA evolution has been necessary for addressing anthropological questions and clinical studies addressing age and origin of Africans, Europeans, Asians, and Native Americans (refer to Table 7.0 for mtDNA haplogroups). Two major databases regarding human mitochondrial genome and variations are: (1) MITOMAP: Human Mitochondrial Genome Database and (2) GiiB-JST mtSNP database: Human Mitochondrial Genome Polymorphism Database (Umetsu and Yuasa, 2005). Studies investigating the role of various mitochondrial haplogroups in the pathogenesis of MCD are scarce.

Oxidative stress is one of the major risk factors for neurodegenerative diseases. Mitochondrial DNA (mtDNA) variations can cause inefficient oxidative phosphorylation leading to the accumulation of ROS, DNA damage, and may lead to increased brain lesion risk. Since the roles of various mtDNA variations in the pathogenesis of MCD are scarce. Studies exploring the presence and the roles of mitochondrial haplogroup background and mtDNA variations as risk contributors to MCD are needed. Overall, the goal of this study is to investigate the mtDNA background and oxidative damage in intractable pediatric epilepsy patients with MCD phenotype.

Table 1.0.
Cortical dysplasia classification in epilepsy

Type of Focal Dysplasia	Subtype	Major morphological feature(s)
Mild MCD	Type I	Excess neurons in layer I
	Type II	Excess neurons outside layer I
FCD Type I	Type Ia	Cortical dislamination only
	Type Ib	Cortical dislamination and giant neurons
FCD Type II (Taylor-Type FCD)	Type IIa	Cortical dislamination and dysmorphic neurons
	Type IIb	Cortical dislamination, dysmorphic neurons and balloon cells

Table 2.0.
Classification of Malformations of Cortical Development (MCD)

Malformations due to abnormal glial and neuronal proliferation	Dysembrioplastic neuroepithelial tumors (DNET) Focal cortical dysplasia (FCD) Fukuyama muscular dystrophy syndrome (FMDS) Gangliomas Hemimegalencephaly Microcephaly Microlissencephaly Megalencephaly
Malformations due to abnormal neuronal migration	Lissencephaly Cobblestone dysplasia Periventricular nodular heterotopia (PNH)
Malformations due to abnormal cortical organization	Polymicrogyria Schizencephaly
Malformations due to abnormal cell fate proliferation	Tuberous sclerosis (TS)

Table 3.0.
Susceptibility Genes of MCD Syndromes

MCD	Gene	Locus	Protein
Tuberous Sclerosis (TS)	*TSC1*	9q34	Hamartin
Tuberous Sclerosis (TS)	*TSC2*	16p13	Tuberin
X-linked lissencephaly (XLIS)	*DCX*	Xq22	Doublecortin (DCX)
Subcortical band heterotopia**	*DCX*	Xq22	Doublecortin (DCX)
Miller-Dieker lissencephaly	*LIS-1*	17p13	PAFAH1B1***
Fukuyama congenital muscular dystrophy (FCMD)	*FCMD*	9q31	Fukutin
Muscle-eye-brain disease (MEB)**	*POMGnT1*	1p32	POMGnT1***
Periventricular nodular heterotopia (PH)	*FLN1*	Xq28	Filamin 1

**Not discussed in this review
***PAFAH1B1 – platelet activating factor acetyldydrolase β subunit
POMGnT1- protein O-manose β 1,2-N-acetylglucosaminyltransferase

Table 4.0.
Environmental Factors of MCD

Environmental Factor	Agent
Teratogenic	Alcohol Cocaine Methlylazoxymethanol (MAM) Thalidomide
Physical	Freeze lesion Hypoxic-ischemic injury Head Trauma Ionizing radiation Ultraviolet (UV) radiation
Biological	Cytomegalovirus Herpes simplex Rubella Toxoplasmosis

Table. 5.0.
Mitochondrial variants reported in Diseases and Haplogroups

Region	Variant	Reported in Patients
tRNA Phe	G583A	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS)
tRNA Phe	A606G	Exercise intolerance/ myoglobinuria
tRNA Phe	T618C	Mitochondrial myopathy
12S rRNA	G709A	Non-syndromic hearing loss
12S rRNA	T1095C	Sensorineural Hearing Loss (SNHL)
12S rRNA	T710C	Colorectal tumor; mtDNA haplogroup (Hg)-L1b
12S rRNA	C1310T	Diabetes mellitus
12S rRNA	A1555G	Maternally inherited deafness or aminoglycoside-induced deafness
tRNA Val	G1606A	Ataxia, myoclonus, and deafness
tRNA Val	G1642A	MELAS
16S rRNA	G1719A	Hg-I, X
16S rRNA	T1738C	colorectal tumor
16S rRNA	C3093G	MELAS
16S rRNA	G3196A	Alzheimer's Disease and Parkinson's Disease (ADPD)
16S rRNA	T3197C	Hg-U5
tRNA Leu	A3243G	MELAS, (Chronic Progressive External Ophthalmoplegia) CPEO; Diabetes/deafness
tRNA Leu	A3243T	Mitochondrial myopathy
ND1	T3308C	MELAS, colorectal tumor
ND1	T3394C	Leber Hereditary Optic Neuropathy (LHON); Non-insulin dependent diabetes mellitus (NIDDM); acute leukemia
ND1	C3594T	Hg-L(L1 or L2)
ND1	A3397G	ADPD
ND1	A4136G	LHON
tRNA Ile	A4269G	Fatal Infantile Cardiomyopathy (FICP)

Table. 5.0. (cont.)
Mitochondrial variants reported in Diseases and Haplogroups

Region	Variant	Reported in Patients
tRNA Ile	T4274C	CPEO
tRNA Ile	A4295G	Maternally inherited hypertropic cardiomyopathy (MHCM)
tRNA Ile	G4298A	CPEO / Multiple Sclerosis (MS)
tRNA Ile	G4309A	CPEO
tRNA Ile	A4317G	Fatal Infantile Cardiomyopathy (FICP)
tRNA Ile	A4320T	Mitochondrial encephalocardiomyopathy
tRNA Gln	T4336C	ADPD
tRNA Met	T4409C	Mitochondrial myopathy
ND2	A4917G	LHON, Hg-T
tRNA Trp	G5521A	Mitochondrial myopathy
tRNA Trp	G5549A	Dementia/ chorea
tRNA Ala	T5628C	CPEO
tRNA Asn	A5692G	CPEO
tRNA Cys	A5814G	Mitochondrial encephalopathy
tRNA Tyr	T5874G	Exercise intolerance
tRNA Ser(UCN)	A7445G	SNHL
tRNA Ser(UCN)	A7497A	Mitochondrial myopathy
tRNA Ser(UCN)	T7511C	SNHL
COX II	G8251A	SNHL; Hg-I,W
ATP6	G8994A	SNHL; Hg-W
ATP6	G9055A	Hg-K, longevity, ↓PD
CO III	G9438A	LHON
CO III	G9738T	LHON

Table. 5.0. (cont.)
Mitochondrial variants reported in Diseases and Haplogroups

Region	Variant	Reported in Patients
CO III	G9804A	LHON
CO III	G9952A	Mitochondrial Encephalopathy
CO III	T9957C	Progressive encephalopathy (PEM); MELAS
tRNA Gly	T9997C	MHCM
tRNA Gly	A10006G	Chronic intestinal pseudo-obstruction (CIPO)
tRNA Gly	T10010C	PEM
tRNA Gly	T10034C	Hg-I
tRNA Gly	A10044G	Gastroesophageal reflux (GER) / Sudden infant death syndrome (SIDS)
ND3	A10398G	↓PD, ↓AD;A-↑Breast Cancer (BRCA) in AA; Hg-I, J, L, M
ND3	C10400T	Hg-M
tRNA LeuCUN	A12308G	Hg-U&K; CPEO / Stroke / Cardiomyopathy (CM)
ND5	G13368A	Hg-T
ND5	G13708A	Hg-J; LHON
tRNA Thr	G15915A	Mitochondrial myopathy
tRNA Thr	A15923G	Lethal infantile mitochondrial myopathy

Table 6.0.
Mitochondrial haplogroups

Haplogroup	Diagnostic mtDNA Variation (SNP)
A	A663G
B	8280:8290 =A[delCCCCCTCTA]G
C	A13263G
D	C5178A
E	C13626T
F	T6392C
G	A4833G
H	C7028C
I	A4529T , T10034C, A10398G
J	A10398G and G13078A
L0, L1, L2	C3594T
L3	C3594C
M	C10400T + A10398G
N	C10400C + A10398A and T10873T
P	T10118C
Q	A5843G
R	C12705C
S	T8404C
T	A4917G
V	G4580A
W	A11947G
X	C6371T
Y	G8392A
Z	T9090C
HV-group	C14766C
TJ-group	T4216C
UK-group	A12308G and G9055A

Table 7.0.
mtDNA lineage

Ancestry	Haplogroups
African	L0, L1, L2, L3, M, and N
Asian	A, B, C, D, E, F, G, M, N P, Q, Y, and Z
European	F, B, H, Hv, I, J, K, P, R, T, U, V, X, and W
Native American	A, B, C, and D
Siberia	G, Y, and Z

CHAPTER III

MANUSCRIPT 1

MITOCHONDRIAL DNA BACKGROUND AND OXIDATIVE DAMAGE IN INTRACTABLE EPILEPSY PEDIATRIC PATIENTS

Abstract

Objectives: Research mitochondrial background and mitochondrial DNA (mtDNA) damage in pediatric intractable epilepsy patients. mtDNA oxidative damage and copy number are indices of mitochondrial damage. Mitochondrial damage may play a role in the pathology of intractable epilepsy. The purpose of this study is to determine and compare mtDNA variants (SNPs) and mtDNA oxidative damage in intractable epilepsy patients with malformations of cortical development (MCD) and non-MCD with non-epileptic controls.

Methods: Brain tissue specimens were collected from 21 pediatric intractable epilepsy patients from Miami Children's Hospital and 11 controls (non-epileptic) from UMB. Oxidative mtDNA damage as indicated by $mtDNA^{\Delta Ct}$ (formation of 8-OHdG) and relative mtDNA copy number were determined for each tissue by quantitative real-time PCR (QPCR). A total of 10 SNPs associated with mitochondrial myopathies were genotyped by allele-specific oligonucleotide dot (ASO) blot analysis. In female samples, a summary Bayesian network was created to investigate the relationship of these variables.

Results: In female samples, relative mtDNA copy number were higher in intractable epilepsy patients compared to non-epileptic control samples (p=0.01). Oxidative mtDNA damage was lower in epileptics compared to non-epileptic control samples (p=0.24), and lower in MCD compared to non-MCD (p=0.58). mtSNP G9952A was found in higher frequencies in epilepsy samples. Bayesian network showed several significant relationships ($p < 0.05$) between epilepsy, MCD, oxidative mtDNA damage, mtDNA copy number, and the mtSNPs G9952A, G3196A, T3197C, A10006G, and A10398G in female samples.

Conclusion: These data suggest that the mtSNPs explored are associated with intractable epilepsy phenotypes. mtDNA copy number and $mtDNA^{\Delta Ct}$ may serve as useful biomarkers of mtDNA damage and can be used to evaluate mitochondrial oxidative damage in epilepsy etiologies. These results indicate that increases in mtDNA content may be compensatory mechanisms for defective mitochondria in intractable epilepsy. Findings suggest mitochondria play a role in the development of epilepsy. Thus, means to suppress oxidative damage may be beneficial to intractable epilepsy patients.

Epilepsy is a chronic neurological disorder characterized by spontaneous recurring seizures. The incidence of epilepsy is highest for children and the elderly. A proportion of patients have seizures that are resistant to traditional anti-epilepsy medicines (intractable or refractory epilepsy) (Alexander and Godwin, 2006). Over 30% of epileptic patients are medically intractable (Lopez et al., 2007). The occurrence of epilepsy is highly associated with malformations of cortical development (MCD) which are developmental brain lesions that consist of dysplastic neuronal lesions (malformations) (Schwartzkroin and Walsh, 2000; Hua and Crino, 2003; Hader et al., 2004; Rickert, 2006; Wong, 2007). MCD are increasingly being recognized as the cause of intractable epilepsy.

MCD presents a broad spectrum of structural changes which appear to result from changes in precursor neuronal or neuronal cells during cortical development at various stages such as: proliferation, migration, differentiation, and apoptosis (Becker et al., 2004). Treatment with anti-epileptic drugs (AEDs) is usually ineffective, and children with MCD may require surgical removal of the affected area of the brain (Yasin et al., 2010). Two of the most common MCD found in resected tissue from children afflicted with intractable epilepsy are tuberous sclerosis (TSC), and focal cortical dysplasia (FCD) (Fassunke et al., 2004). The molecular mechanisms underlying the formation of MCD are still largely unknown and the treatments for epilepsy due to MCD are often ineffective or limited (Wong, 2009).

Epilepsy results from excessive synchronous firing of neurons in cortical networks (Prasad et al., 1999; Paredes and Baraban, 2002). Despite several attempts to elucidate the cause of epilepsy, to date, results have not been satisfactory. Epilepsy is a

common feature of mitochondrial encephalopathies that are caused by defective oxidative phosphorylation in the CNS (Zsurka et al., 2010). It has been proposed that the accumulation of mitochondrial DNA (mtDNA) mutations and the alteration in the execution of apoptosis, contribute to the onset of neurodegenerative diseases (Lee et al., 2005). A role for mitochondrial dysfunction and oxidative stress in intractable epilepsy patients has been suggested (Shah et al., 2002; Waldbaum and Patel, 2010a).

Mitochondria contain their own genome, mtDNA, which consists of a 16.5-kb circular double-stranded DNA (dsDNA) molecule containing 37 genes (Bai et al., 2008). Mitochondria functions in oxidative phosphorylation, heme, lipid, amino acid biosynthesis, and fatty acid oxidation among other functions (Sugimoto, 2008). Mammalian cells contain several hundreds to more than a thousand mitochondria. The size, shape and abundance of mitochondria vary drastically in different cell types and may change under differing energy demands and physiological or environmental conditions. In a cell, the abundance of mitochondria is determined by biogenesis and division of the organelles. The quantity of mitochondria per cell is tighly regulated by activation of specific transcription factors and signaling pathways (Lee and Wei, 2005). The major source of ATP in cortical neurons is provided by mitochondrial oxidative phosphorylation (Chuang, 2010). Mitochondrial metabolism also generates the majority of the reactive oxygen species (ROS) production in cells (St-Pierre et al., 2006). ROS results when unpaired electrons escape the electron transport chain. The most commonly produced base lesion by ROS, and the most often measured as an index of oxidative DNA damage is 8-hydroxyguanosine (8-OHdG) (Wiseman and Halliwell, 1995). The brain is believed to be particularly susceptible to the damaging effects of ROS damage

due to its high metabolic rate and reduced capability for cellular regeneration compared to other organs (Anderson, 2004). Mitochondria can modulate neuronal excitability and synaptic transmission since oxidative phosphorylation provides the major source of ATP in neurons and participate in cellular calcium homeostasis (Devi et al, 2008).

Mitochondrial oxidative stress and dysfunction are contributing factors to several neurological disorders (Shokolenko et al., 2009; Waldbaum and Patel, 2010b). Encephalomyopathies have been found in children in association with defects in mitochondrial structure and function. Some of these disorders are acute leukemia, Alzheimer's disease (AD), cardiomyopathy (CM), chronic progressive external ophthalmoplegia, colorectal cancer, deafness, fatal infantile cardiomyopathy (FICP), gastroesopageal reflux (GER), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), Leber Hereditary Optic Neuropathy (LHON), Parkinson's disease (PD), maternally inherited hypertropic cardiomyopathy (MHCM), multiple sclerosis (MS), non-insulin dependent diabetes mellitus (NIDDM), non-syndromic hearing loss, progressive encephalopathy (PEM), sensorineural hearing loss (SNHL), strokes, and sudden infant death syndrome (SIDS) (Filiano et al., 2002; Bai et al., 2007; Tzen et al., 2007). Among mitochondrial disorders, infantile spasms have been reported in complex III deficiency (Shah et al., 2002). In this study, we investigate the presence of oxidative mtDNA damage, constitutional mtDNA copy number, mtDNA SNPs, and haplogroups as sources of genetic diversity that may contribute to the development of pediatric intractable epilepsy.

Methods

Collection of brain tissue from pediatric intractable epilepsy patients

Brain tissue samples from 21 pediatric non-neoplastic intractable epilepsy patients who have undergone brain resection surgery at the Miami Children's Hospital, Miami, FL, during 2008-2009 were collected. Tissues obtained during surgical resection were immediately snap-frozen in liquid nitrogen and stored at -80°C. As references, eleven normal, non-epileptic, pediatric brain tissues were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD and stored at -80°C. This study was approved by the Florida International University's Institutional Review Board.

DNA extraction

Freshly excised human neuronal (brain) tissue was stored in liquid nitrogen and frozen in -80°C until ready for processing. The frozen neuronal tissue was homogenized, while on ice, using a Janke and Kunkle TP-18-10 blade type homogenizer in which 1 ml of Trizol® was added. The homogenate was transferred to a 2.0-ml microcentrifuge tube. DNA was then isolated and purified via Phenol-Chloroform extraction. The precipitated DNA was pelleted and resuspended in1X TE buffer. The integrity of the DNA was verified following electrophoresis through 2% agarose gels.

Determination of and mtDNA copy number and mitochondrial DNA oxidative damage by quantitative real time PCR

Quantitative real-time PCR (QPCR) assays were performed using Applied Biosystems 7300 System with a final volume of 25uL reaction mixture containing 50ng DNA template, 12.5uL SYBR Green PCR Master Mix (Qiagen), and 10mM of each

primer. The sequences for the primers used for the amplification of the mitochondrial gene *NADH Dehydrogenase 1* (*ND1)* were: mtF3212, 5'-CACCCAAGAACAGGGTTTGT-3' and mtR3319, 5'-TGGCCATGGGATTGTTAA-3'. The sequences of the primer for the nuclear housekeeping gene *18s rRNA*, used for the normalization in the QPCR analysis, were: 18s1546F, 5'-TAGAGGGACAAGTGGCGTTC-3'and 18s650R, 5'-CGCTGAGCCAGTCAGTGT-3' (Bai et al., 2004; Lin et al, 2008). The QPCR conditions were set up as follows: hot start at 95° C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The fluorescence intensity was measured at the end of the extension phase at 60°C. All samples were performed in duplicated and a non-template negative control was included in each reaction.

A total of 50ng of DNA was used in QPCR for the determination of the threshold cycle number (Ct) of the *18s rRNA* and *ND1* genes. Ct values can be used as a measure of input copy number and the Ct value differences were used to quantify the mtDNA copy number relative to the 18s rRNA gene with the following equation:
Relative copy number= $2^{\Delta Ct}$, where ΔCt is the Ct *18s rRNA* – Ct *ND1* (Szuhai et al., 2001; Bai et al., 2004; Lewis et al., 2007; Edwards, 2009; Lee et al, 2010).

Since degree of oxidative mtDNA damage is reflected by an abundance of 8-OHdG formation in mtDNA, the content of 8-OHdG in mtDNA, an index for cellular oxidative damage, was determined by QPCR and presented as ΔCt (Ayala-Torres et al., 2000). As indicated by Lin et al (2008), the amplification efficiency would decrease after treatment of the DNA sample with hOGG1 to remove the 8-OHdG residue to form an abasic site. The content of 8-OHdG in the samples' mtDNA was determined by treating

the 200ng sample with 2 units of hOGG1 (New England Biolabs, M024S) at 37° C for 1 hour and 5 minutes to remove the 8-OHdG residue to form a basic site. The digested mtDNA were amplified by QPCR using the primers for *ND1* gene. PCR amplification efficiencies of DNA templates containing a single 8-OHdG or two 8-OHdGs at least 13 base pairs apart are not significantly disturbed (Ct_1), however, the presence of an abasic site in DNA after treatment of hOGG1 would dramatically reduce the PCR efficiency, thus, increasing the Ct value (Ct_2) (Lin et al., 2008). The degree of oxidative mtDNA damage, $mtDNA^{\Delta Ct}$, was defined as $\Delta Ct = Ct_2$ (hOGG1 treatment) – Ct_1 (no hOGG1 treatment) (Lin et al., 2008; Su et al., 2010). Each analysis was performed in duplicate, and the mean value of ΔCt was calculated for each sample. Hence, the larger the ΔCt, the more abundant the 8-OHdG and more oxidative damage present in the sample.

mtDNA genotyping

A total of 50ng of DNA was used in Quantitative PCR (QPCR) to amplify the mitochondrial genome with 19 primer sets (Table A.8) designed by Bai et al. (2007) to include the mtDNA regions containing 10 reported mtDNA variations (SNPs). The mtDNA variations are distributed along the rRNA, tRNA, COIII and ND3 regions of the mitochondrial genome. The mtDNA variations studied have been reported in patients with varying diseases such as: Alzheimer's disease, Parkinson's disease, Leber's hereditary optic neuropathy (LHON), deafness, chronic progressive external ophthalmoplegia (CPEO), chronic intestinal pseudo-obstruction (CIPO) and other diseases (Table 1.3).

QPCR assays to amplify 19 regions of the mitochondrial genome (Table A.8) were performed using Applied Biosystems 7300 System with a final volume of 25uL

reaction mixture containing 50ng DNA template, 12.5uL SYBR Green PCR Master Mix (Qiagen), and 10mM of each primer. The QPCR conditions were set up as follows: hot start at 95° C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Two microliters of QPCR products were spotted on Hybond N+ membrane (GE). Dot blot preparation and hybridization conditions are available in the Appendix (Table A.11). The allele-specific oligonucleotide (ASO) probes for the mtDNA variants are listed in Table A.9 (Bai et al, 2007). These probes were labeled with Dioxigenin (DIG) (Roche, DIG Oligonucleotide 3'-End Labeling Kit). Both the variant and wild type controls for each ASO blot were included as controls. mtDNA haplogroups I, J, L, M and U5 were classified according to Table A.7.

Bayesian network

In female samples, Bayesian Network Inference with Java Objects (Banjo) © software was used to determine influence scores and to create a directed acyclic graph (DAG) to represent the Bayesian network showing the probablilistic relationships between epilepsy status, MCD status, relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs with influence scores (absolute value) higher than 0.1. The top three scoring networks were used to generate a consensus network.

Statistical Analysis

Continuous variables were studentized to identify and remove outliers. Logarithmic transformation of data was used since the original values of the relative mtDNA copy number and the content of 8-OHdG in mtDNA showed non normal distributions. The continuous variables between groups were compared using the Student's t-test and Fisher's exact test for comparisons of the frequency of dichotomous

features. Pearson's correlation test was used to test the relationship between mtDNA copy number and the content of 8-OHdG in mtDNA. Analysis was carried out using Fisher's exact test for each individual mtSNP and haplogroup.

Logistic regression was used to determine the adjusted odds ratio. Epilepsy patients were sub-divided as those being malformations of cortical development (MCD) and non-MCD as per Barkovich MCD classification system (Barkovich et al., 2005). Logistic regression was also used to explore if epilepsy, MCD, or non-MCD phenotypes were dependent on mtSNPs, haplogroups, level of mtDNA oxidative damage, and level of relative mtDNA copy number, model adjusted by age. In female samples, Banjo © software was used to determine influence scores and to create a directed acyclic graph (DAG) to represent the Bayesian network showing the probablilistic relationships between epilepsy status, MCD status, relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs. In addition, Pearson correlations were determined for these relationships. Statistical analyses were performed using SPSS version 18.0 for Microsoft Windows. Statistical significance was set at $P < 0.05$.

Results

Demographics and clinical information

The demographic and pathology information of the pediatric non-neoplastic intractable epilepsy patients are shown in Table 1.1, and for the 11 control samples in Table 1.2. After the removal of outliers there were 18 pediatric non-neoplastic epilepsy patients (MCD=12, non-MCD=6). MCD cases accounted for ~66% of the non-neoplastic intractable epilepsy cases. The majority (92%) of patients with MCD are classified as malformations due to abnormal glial and neuronal proliferation. The groups did not

significantly differ in ages. Using correlation methods, we demonstrate that mtDNA copy number and the degree of oxidative DNA damage were not significantly correlated (Pearson correlation= -0.28, p=0.19) (Fig. 1.1).

Relative mtDNA copy number

The measurement of DNA in individual amplifiable DNA segments is measured by quantitative real-time PCR (QPCR). The use of SYBR green in QPCR assays allows for the detection of small difference in the starting template using nanograms of DNA. The higher threshold cycle (Ct), or shift of the amplification curve to the right, indicates a low amount of starting DNA template, thus, decreased DNA content. Ratios of mtDNA/nuclear DNA were used to obtain the relative mtDNA copy number, where a lower ratio is representative of a lower initial DNA template, demonstrating a decrease in the amount of mtDNA. We selected the nuclear *18s rRNA* gene and mtDNA coding region of *ND1* gene. We used QPCR to analyze the relative mtDNA copy number of the pediatric intractable epilepsy brain tissues (non-neoplastic) and non-epileptic control brain tissues. Tables 1.4 - 1.8 show the average relative mtDNA copy number for each group. The relative mtDNA copy number (p=0.44) did not significantly differ between male and female epilepsy patients (Table 1.5). A 70% increase in relative mtDNA copy number for non-neoplastic epilepsy samples was observed compared to controls ($p < 0.001$). Both MCD (2.34, $p < 0.001$) and non-MCD (2.60, p=0.02) samples demonstrated higher relative mtDNA copy numbers compared to controls (1.33). MCD patients presented a lower relative mtDNA copy number (2.34) than non-MCD (2.60) epileptic patients, however, statistical significance was not achieved (p=0.58). Higher

relative copy numbers were found in female non-neoplastic epilepsy samples compared to controls (p=0.01).

Oxidative mtDNA damage

The degree of oxidative mtDNA damage is reflected by an abundance of 8-OHdG formation in mtDNA, the content of 8-OHdG in mtDNA, an index for cellular oxidative damage, was determined by QPCR and presented as ΔCt. The degree of oxidative mtDNA damage, $mtDNA^{\Delta Ct}$, was determined as $\Delta Ct = Ct_2$ (hOGG1 treatment) – Ct_1 (no hOGG1 treatment), thus, the larger the ΔCt, the more abundant the 8-OHdG and more oxidative damage present in the sample. Tables 1.4 -1.8 show the oxidative mtDNA damage for each group. Controls had higher oxidative mtDNA damage (0.59) compared to non-neoplastic epilepsy (0.43, p=0.47) and MCD (0.30, p=0.28), but lower oxidative mtDNA damage compared to non-MCD (0.77, p=0.21) groups, yet, statistical significance was not reached. The degree of oxidative mtDNA damage (p=0.35) did not significantly differ between male and female epilepsy patients, and female epilepsy samples presented lower oxidative mtDNA damage than controls (p=0.24) (Table1. 5).

mtDNA genotyping

In this study we analyzed the association between ten mitochondrial SNPs and non-neoplastic intractable epilepsy (MCD and non-MCD). Alleles G9804A (p=0.51; Adjusted OR=1.27E9, 95% CI [0, NaN]) and G9952A (p=0.27; Adjusted OR=1.48E9, 95% CI [0, NaN]) were found at higher frequencies in epilepsy samples compared to controls. Controls had higher frequencies for the mitochondrial SNPs G3196A, T3197C, A10006G, A10398G, and the haplogroups I, J, L, M, and U5 compared to all non-neoplastic epilepsy samples (Tables 1. 9 and 1.10), including MCD (Tables 1.15 and

1.16) and non-MCD (Tables 1.12 and 1.13) sub-groups. The European (I and J), African (L and M), and Asian (M) haplogroups were observed in both epilepsy and control samples. The European haplogroup (U5) was not observed in the epilepsy (MCD and Non-MCD) samples. However, no differences in the frequencies of SNPs and haplogroups were observed when comparing MCD and Non-MCD sub-groups (Tables 1.19 and 1.20). The same trends were observed when comparing female epilepsy samples with controls. In addition, logistic regression revealed that epilepsy, MCD or non-MCD phenotypes did not significantly depend on level of relative mtDNA copy number, level of oxidative mtDNA damage, mtSNP or haplogroup status (Tables 1.21-1.26). A trend that the levels of relative mtDNA copy number and oxidative mtDNA damage and for the SNPs A4317G (OR= 1.00E8, 95% CI [0, NaN]) and T10010 (OR= 1.69E38, 95% CI [0, NaN]) predicted epilepsy phenotype was observed, yet, there were large confidence intervals due to large standard error (Table 1.21).

Bayesian network

In the female samples we used Banjo© software to learn a Bayesian network to study the relationships among epilepsy status, MCD status, relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs. A summary Bayesian network based on the top three Bayesian networks for the female samples was created (Figure 1.2). In this network epilepsy status is connected to oxidative mtDNA damage, relative mtDNA copy number, and to 6 mtDNA SNPs (T3197C, G3196A, A10398G, A10006G, G9952A, and A1555G). The paths of oxidative mtDNA damage-A10006G-epilepsy; oxidative mtDNA damage-relative mtDNA copy number; epilepsy- relative mtDNA copy number; G9952A-epilepsy; epilepsy-A103098G, T3197C, G3196A, and A1555G are

shown in the summary Bayesian network. The influence scores between several of these variables are depicted on Table 1.27. The Pearson correlation of between selected variables based on the influence scores are found in Table 1.28. Signficant correlations ($p < 0.05$) were found between epilepsy and MCD status, epilepsy and MCD with G9952A, epilepsy and MCD with oxidative mtDNA damage by relative mtDNA copy number, epilepsy and MCD with T3197C, G3196A, A10006G, and A10398G, to name a few. Thus, the summary Bayesian network summarizes the relationship between mtDNA oxidative damage and copy number, SNPs (genes) and disease (phenotype).

Discussion

A role for mitochondrial dysfunction and oxidative stress in intractable epilepsy patients has been suggested (Shah et al., 2002; Waldbaum and Patel, 2010a). Results of this study suggest mitochondria may play a critical role in the development of epilepsy. In this study we demonstrated that pediatric non-neoplastic intractable epilepsy patients (both MCD and non-MCD syndrome) have significantly higher mtDNA copy number than controls. Female epilepsy samples presented signficantly higher mtDNA copy number and a trend of lower oxidative mtDNA damage compared to controls. The degree of oxidative mtDNA damage was lower among epilepsy patients, including MCD, compared to controls. No significant differences by gender, regarding relative mtDNA copy number and degree of oxidative mtDNA damage, as well as mtDNA SNP and haplogroup background, were observed among pediatric epilepsy patients. The mtDNA variants G9804A and G9952A, which code for COIII, were found in higher frequencies in the intractable epilepsy patients. A trend in which G9804A and G9952A, along with the level of mtDNA copy number and the level of oxidative mtDNA damage, predict

MCD and non-MCD epilepsy phenotypes was observed. In the female samples G9952A was found in higher frequency than controls. In addition, in female samples, the summary Bayesian network demonstrated the statistically significant relationships between the oxidative mtDNA damage, relative mtDNA copy number, SNPs (T3197C, G3196A, A10398G, A10006G, G9952A, and A1555G), and epilepsy. Additionally, the majority of the epilepsy patients were categorized as MCD that may be further classified as malformations due to abnormal glial and neuronal proliferation. A key event in brain development is proliferation which begins around the 20th week of gestation (Lenroot and Giedd, 2006).

Mammalian cells contain several hundreds to more than a thousand mitochondria. The size, shape and abundance of mitochondria vary drastically in different cell types and may change under differing energy demands and physiological or environmental conditions. In a cell, the abundance of mitochondria is determined by biogenesis and division of the organelles. The quantity of mitochondria per cell is tightly regulated by activation of specific transcription factors and signaling pathways (Lee and Wei, 2005). The assembly and functioning of the respiratory enzyme complexes in cells require coordinated expression the interaction of gene products between the mitochondrial and nuclear genomes. Gene expressions of the mitochondria and nucleus respond in a complex manner to various physiological and developmental signals such as growth activation, neoplastic transformation, muscle contraction, cell differentiation and hormone action (Lee and Wei, 2005). Control of biogenesis in mitochondria is a complex process. Alterations in intracellular level of ROS are associated with changes in mitochondrial abundance, mtDNA copy number, and the expression of respiratory genes.

Persistent oxidative stress in mitochondria not only contributes to the somatic mtDNA mutations but also alter mtDNA replication rate, leading to an overall decline in the mitochondrial respiratory function.

Replication of mtDNA occurs primarily during the S and G_2 phases of the cell cycle, but most importantly can occur at any point of the cell cycle. In addition, mtDNA replication does not occur concurrently with the growth and division of organelles, hence, mtDNA replication can occur without mitochondrial proliferation. The copy number of mtDNA varies with cell type and is maintained within a range. mtDNA copy number may be modulated according to the energy needs of the cell. Changes in mtDNA copy number in response to exercise and hormone treatment have been observed (Lee and Wei, 2005). Currently, it is unclear how copy number of mtDNA and the abundance of mitochondria are regulated under different physiological and developmental conditions.

mtDNA copy numbers can be modulated when physiological conditions are changed. Environmental exposures can generate ROS and may induce the accumulation of mtDNA mutations in human tissue. mtDNA is more susceptible to oxidative damage due to its lack of histones and limited capacity to repair DNA damage, thus, consequently acquires mutations at greater rates than nuclear DNA. Mitochondrial function is compromised as a result of oxidative damage and damage to mtDNA. Oxidative stress stimulates mitochondrial proliferation to meet the energy needs for cell survival including repair of damage and synthesis of new proteins. Oxidative stress causes excess ROS production resulting in further oxidative damage. The increase of mtDNA copy number is dependent of the level of oxidative stress, the capacity of intracellular antioxidant system, the quality of mitochondria and mtDNA. The increase of ROS production from a

defective respiratory chain is thought to play a role in the increase of mitochondrial content. ROS act as a second messenger to trigger the expression of nuclear respiratory factors and mitochondrial transcription factor to induce mitochondrial biogenesis and mitochondrial proliferation (Jiang et al., 2005). Oxidative damage induces an increase in mitochondria and mtDNA to compensate for the decline in the function of mitochondrial respiration (Shen et al., 2008). In other words, ROS resulting from oxidative stress interact with genetic signaling systems that upregulate gene expression to counteract stressor challenges and to re-establish hemeostatis. Increase in mtDNA copy number has been found in diseases.

The mtDNA copy number in leukocytes from patients aged 30 years and younger with MELAS and MERFF showed an increase in mtDNA copy number, and a depletion of mtDNA was found in patients 40 years and older (Liu et al., 2006). Brinckmann et al. (2010), found increased mtDNA copy number in the brain tissue of a 16 year old girl with MERRF. According to Lee and Wei (2005), increase in mtDNA copy number is associated with elevated levels of oxidative stress in the aging tissues, brain, lung, and skeletal muscle of aged individuals. Chen et al. (2007) found increased total mtDNA copy number in peripheral leukocytes in Huntington's disease patients, and the transcription levels of mtDNA-encoded enzymes were not significantly elevated. Chen et al. (2007) suggested that the oxidative damage to mtDNA in HD leukocytes has reached a threshold over which mtDNA-encoded mRNA expression was suppressed.

Treatment of human cells with H_2O_2 and buthionine suphoximine, which deplete intracellular glutathione, an anti-oxidant, induces an increase in mtDNA copy number and mitochondrial mass (Lee et al., 2000). Gamma-irradiated mice showed an increase in

relative mtDNA copy number in brain and spleen tissues, suggesting that the major mechanisms for maintenance of the mitochondrial genome is the induction of synthesis of new mtDNA copies because the repair systems in the mitochondria function at a low level efficiency (Malakhova, et al., 2005). It has been suggested that increases of mtDNA copy number in aging tissue cells are a result of the feedback response that compensates for defective mitochondria bearing impaired respiratory chain or mutated mtDNA. Mitochondrial copy number may be an influence on phenotypic expression.

There have been several reports showing that oxidative stress may be an important mechanism of CNS damage. Findings by Fukuda et al. (2008) suggest that oxidative stress plays an important role in brain damage in children and this damage correlates with disease state. For example in status epilepticus excitatory glutamic acids released in massive amounts might injure neurons and the production of free radicals by excessive stimulation of excitatory amino acid receptors are considered to be the cause of epilepsy (Fukuda et al., 2008). Increased oxidation by ROS in cellular macromolecules after prolonged seizures has been observed, and experimental seizures have shown impaired Ca^{2+} sequestering, excessive ROS production, increased nitric oxide and peroxynitrite generations after prolonged seizures at time points preceding neuronal death (Waldabaum and Patel, 2010). Oxidative stress and mitochondrial dysfunction occur as a consequence of prolonged epileptic seizure and may contribute to seizure-induced brain damage (Patil et al., 2011).

Temporal lobe epilepsy studies have suggested that preceding neuronal death, after status epilepticus, is the increased level of ROS observed in brain slices and slice cultures of several models of experimental epilepsy, such as kainite-induced hippocampal

damage or pilocarpine damage (Baron et al., 2007). Concentrations of 8-OHdG in the cerebral spinal fluid (CSF) in samples from children with epilepsy were slightly higher than control group, but the differences were not statistically significant (Fukuda et al., 2008). Shokolenko et al. (2009) provided evidence that in the human colon carcinoma (HCT116) cell model treated with H_2O_2, both DNA repair and degradation processes operate on oxidatively damaged mtDNA. They found that the elimination of damaged mtDNA is followed by an accumulation of linear mtDNA molecules, which are thought to represent degradation intermediate, that unlike undamaged circular mtDNA molecules, they are susceptible to exonucleolitic degradation. Shokolenko et al. (2009) suggest that trends that if a cell is unable to repair all of the damage inflicted by the environmental insult (H_2O_2) in the mitochondrial genome, a fraction of the mtDNA molecules undergoes double-strand breaks and is degraded, while moderately damage genomes are repaired, reflected by the increase in mtDNA quantity. Hence, the model presented by Shokolenko et al. (2009) provides a mechanistic explanation, in which moderately damaged mitochondrial genomes are repaired, for the observation that non-neoplastic MCD pediatric epilepsy patients presented a low oxidative mtDNA damage, as indicated by the low $mtDNA^{\Delta Ct}$ (formation of 8-OHdG), and increased mtDNA copy number.

Overall, in our results, we interpret the increase in relative mtDNA copy number as a result of compensatory responses induced by mtDNA damage, in order to repress the actions of the oxidative damage. The increase in mtDNA replication, as a response to oxidative damage, provides a propagation of mtDNA that has not been damaged by oxidative stress. The resulting mtDNA copies do not contain the damage (DNA adducts), presenting an overall lower oxidative mtDNA damage. Thus, the increase in undamaged

mtDNA dilutes the presence of damage mtDNA. Understanding oxidative-stress induced alterations in mtDNA copy number is important for the development of novel drugs to prevent and treat intractable epilepsy.

In addition, our study found that the mitochondrial SNP, G9952A, was found in the female epilepsy patients and not the control samples. This mitochondrial variant codes for COIII. It is believed that the majority of the ROS are generated by complexes I and III, likely due to the release of electrons by NADH and FADH into the ETC. Hanna et al. (1998) identified the G9952A point mutation in a patient with encephalopathy and exercise intolerance. This point mutation is located in 3' end of the gene for the subunit of COIII and is thought to result in the loss of the last 13 amino acids of the C-terminal region of this subunit. Epilepsy has been reported in only a few cases of LHON (Kudin et al., 2009; Niehusman et al, 2011). Our results suggest that the presence of these mitochondrial mutations in COIII, in conjunction with environmental insults, may induce mitochondrial dysfunction in epilepsy. Hence, the role and mechanisms dysfunction of COIII of the respiratory chain in epileptogenesis and MCD needs to be explored further. In addition, the Bayesian network shows a relationship between mitochondria, mtSNP G9952A, and epilepsy phenotype.

In summary, our study shows increased relative mtDNA copy number in brain tissues from pediatric non-neoplastic epilepsy patients. The use of a summary Bayesian network of the female samples demonstrated the statistically significant relationships between the oxidative mtDNA damage, relative mtDNA copy number, SNPs (T3197C, G3196A, A10398G, A10006G, G9952A, and A1555G), and epilepsy. Additionally, the majority of epilepsy patients belonged to MCDs due to abnormal glial and neuronal

proliferation indicating that environmental insults during this process of brain development should be explored. The strength of this study is the direct measurements of relative mtDNA copy number, oxidative mtDNA damage, and genotyping for mtSNPs from the lesioned brain tissues resected from the pediatric epilepsy patients. The summary Bayesian networks for the female samples indicate statistically significant relationships between disease phenotype with several of the mtSNPs, oxidative mtDNA damage, and relative mtDNA copy number. Qualitative changes in mitochondrial genome with specific mutations or deletions in mtDNA have been frequently reported in patients with mitochondrial diseases. However, there are limited studies that have addressed the change in mtDNA copy number in mitochondrial diseases and epilepsy disorders. Many studies have investigated acute consequences of status epilepticus on cellular constituents but less is known about the role of oxidative stress and mitochondrial dysfunction in chronic epilepsy. To our knowledge, this is the first study to explore mtDNA copy number, oxidative mtDNA oxidative stress in MCD pediatric epilepsy patients.

A major limitation of this study is the small sample size. However, results from this study provide the foundation, for proper determination of sample size in pediatric intractable epileptics, such as the frequency of mtSNPs in pediatric epilepsy patients, and the mean and standard deviation for further research regarding measurement of oxidative mtDNA damage. For example, utilizing the results from this study, in order to compare oxidative mtDNA damage between epilepsy (non-tumor) and control samples, and for the study to reach 80% power with an alpha level of 0.05 requires a sample size of 558 (279 disease and 279 control samples). In order to acquire these sample sizes, a multi-facility

(and institution) study is needed to explore the oxidative mtDNA damage, mtDNA copy number, and mtSNPs in children with intractable epilepsy. Findings from this study raise the possibility that inhibition of mitochondrial dysfunction may play a role in successful treatment of epilepsy. Overall, findings of this study indicate a role of mitochondria in epilepsy.

References

Anderson, J.K. (2004). Oxidative stress in neurodegeneration: cause or consequence. *Nature Reviews Neuroscience, 5,* S18-S25.

Alexander, G.M., and Godwin, D.W. (2006). Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy Research, 71,* 1-22.

Ayala-Torres, S., Chen, Y., Svoboda, T., et al. (2000). Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods, 22,* 135-147.

Bai, R., Wong, L., and Leal, S.M. (2008). Mitochondrial DNA variant interactions modify breast cancer risk. *Journal of Human Genetics, 53,* 924-928.

Barkovich, A.J., Kuzniecky, R.I., Jackson, G.D., et al. (2005). A developmental and genetic classification for malformations of cortical development. *Neurology, 65,* 1873-1887.

Baron, M., Kudin, A., and Kunz, W. (2007). Mitochondrial dysfunction in neurodegenerative disorders. *Biochemical Society Transactions, 35,* 1228-1231.

Becker, A.J., Urbach, H., Scheffler, B., Baden, T., Normann, S., et al. (2002). Focal cortical dysplasia of Taylor's balloon cell type: Mutational analysis of the TSC1 gene indicates a pathogenic relationship to tuberous sclerosis. *Annals of Neurology, 52,* 29-37.

Brinckmann, A., Weiss, C., Wilbert, F., et al. (2010). Regionalized pathology correlates with augmentations of mtDNA copy numbers in patients with myoclonic epilepsy with ragged-red fibers (MERRF-Syndrome). *PLoS ONE, 5,* e13513.

Chuang, Y. (2010). Mitochondrial dysfunction and oxidative stress in seizure-induced neuronal death. *Acta Neurologica Taiwanica, 19,* 3-15.

Chen, C., Wu, Y., Cheng, M., et al. (2007). Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. *Biochemical and Biophysical Research Communications, 359,* 335-340.

Devi, P.U., Monocha, A., and Vohora, D. (2008). Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers. *Expert Opinion on Pharmacotherapy, 9,* 3169-3177

Fassunke, J., Blumcke, I., Lahl, R., Elger, C.E., Schramm, J., et al. (2004). Analysis of chromosomal instability in focal cortical dysplasia of Taylor's balloon cell type. *Acta Neurologica, 108,* 129-134.

Filiano, J.J., Goldenthal, M.J., Rhodes, H., et al. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. *Journal of Child Neurology, 17,* 435-439.

Fukuda, M., Yamauchi, H., Yamamoto, H., et al. (2008). The evaluation of oxidative DNA damage in children with brain damage using 8-hydroxyguanosine levels. *Brain and Development, 30,* 131-136.

Hader, W.J., Mackay, M., Otsubo, H., Chitoku, S., Weiss, S., et al. (2004). Cortical dysplastic lesions in children with intractable epilepsy: role of complete resection. *Journal of Neurosurgery: Pediatrics, 100,* 110-117.

Hanna, M., Nelson, P., Rahman, S., et al. (1996). Cytochrome c oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. *American Journal of Genetics, 63,* 29-36.

Howell, N., Oostra, R., Bolhuis, P., et al. (2003). Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber Hereditary Optic Neuropathy. *American Journal of Genetics, 72,* 1460-1469.

Hua, Y., and Crino, P.B. (2003). Single cell lineage analysis in human focal cortical dysplasia. *Cerebral Cortex, 13,* 693-699.

Katayama, Y., Maeda, K., Lizuka, T., et al. (2009). Accumulation of oxidative stress around the stroke-like lesions of MELAS patients. *Mitochondrion, 9,* 306-311.

Kim, M. M., Clinger, B.G., Ha, P.K., et al. (2004). Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clinical Cancer Research, 10,* 8512-8515.

Kudin, A., Zsurka, G., Elger, C., et al. (2009). Mitochondrial involvement in temporal lobe epilepsy. *Experimental Neurobiology, 218,* 326-332.

Kunz, W.S. (2002). The role of mitochondria in epileptogenesis. *Seizure Disorders, 105,* 179-184.

Lan, Q., Lim, U., Liu, C. et al. (2008). A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Neoplasia, 112,* 4247-4249.

Lee, H., Yin, P., Lu, C., et al. (2000). Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical Journal, 348,* 425-432.

Lee, H., Yin, P., Lin, J., et al. (2005). Mitochondrial genome instability and mtDNA depletion in human cancers. *Annals of the New York Academy of Sciences, 1042,* 109-122.

Lee, H., and Wei, Y. (2005). Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *The International Journal of Biochemistry and Cell Biology, 37,* 822-834.

Lee, J., Park, K., Im, J., et al. (2010). Mitochondrial DNA copy number in peripheral blood associated with cognitive function in apparently healthy elderly women. *Clinica Chimica Act, 411,* 592-596.

Lenroot, R., and Giedd, J. (2006). Brain development in children and adolescents: Insights from anoatomical magnetic resonance imaging. *Neuroscience and Biobehavioral Reviews, 30,* 718-729.

Lin, C., Wang, L., Tsai, C., et al. (2008). Low copy number and low oxidative damage of mitochondrial DNA are associated with tumor progression in lung cancer tissues after neoadjuvant chemotherapy. *Interactive Cardiovascular and Thoracic Surgery, 7,* 954-958.

Liu, C., Cheng, W., Lee, C. et al. (2006). Alterations in the copy number of mitochondrial DNA in leukocytes of patients with mitochondrial encephalomyopathies. *Acta Neurologica Scandinavia, 113,* 334-341.

Lopez, J., Gonzalez, M., Lorigados, L., et al. (2007). Oxidative stress in surgically treated patients with refractory epilepsy. *Clinical Biochemistry, 40,* 292-298.

Malakhova, L., Bezlepkin, V., Antipova, V., et al. (2005). The increase in mitochondrial DNA copy number in the tissue of γ-irradiated mice. *Cellular and Molecular Biology Letter, 10,* 721-732.

Niehusmann, P., Surges, R., von Wrede, R., et al. (2011). Mitochondrial dysfunction due to Leber's hereditary optic neuropathy as a cause of visual loss during assessment for epilepsy surgery. *Epilepsy and Behavior, 20,* 38-43.

Paredes, M.F., and Baraban, S.C. (2002). A review of gene expression patterns in the malformed brain. *Molecular Neurobiology, 26,* 1-8.

Patil, C., Ahire, Y., Pathade, P., et al. (2011). Free radical epilepsy and anti-oxidant: An overview. *International Research Journal of Pharmacy, 2,* 64-71.

Pieczenik, S., and Neustadt, J. (2007). Mitochondrial dysfunction and molecular pathways of disease. *Experimental and Molecular Pathology, 83,* 84-92.

Prasad, A.N., Prasad, C., and Stafstrom, C.E. (1999). Recent advances in the genetics of epilepsy: Insights from human and animal studies. *Epilepsia, 40,* 1329-1352.

Rickert, C.H. (2006). Cortical dysplasia: neuropathological aspects. *Child's Nervous System. 22,* 821-826.

Shen, J., Platek, M., Mahasneh, A., et al. (2010). Mitochondrial copy number and risk of breast cancer: A pilot study. *Mitochondrion, 10,* 62-68.

Schwartzkroin, P.A., and Walsh, C.A. (2000). Cortical malformations and epilepsy. *Mental Retardation and Developmental Diagnosis, 6,* 268-280.

Shah, N.S., Mitchell, W.G., and Boles, R.G. (2002). M itochondrial disorders: A potential under-recognized etiology of infantile spasms. *Journal of Child Neurology, 17,* 369-372.

Shen, M., Zhang, L., Bonner, M., et al. (2008). Association between mitochondrial DNA copy number, blood cell counts and occupational benzene exposure. *Environmental and Molecular Mutagenesi, 49,* 453-457.

Shokolenko, I., Venediktova, N., Bockkareva, G.L., et al. (2009). Oxidative stress induces degeneration of mitochondrial DNA. *Nucleic Acids Research, 37,* 2539-2548.

Su, S., Jou, S., Cheng, W., et al. (2010). Mitochondrial DNA damage in spinal and bulbar muscular atrophy patients and carriers. *Clinica Chimica Acta, 411,* 626-630.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J.M., Rhee, J. et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127,* 397-408.

Sugimoto, T., Mori, C., Takanami, T., Sasagawa, Y., Saito, R., et al. (2008). Caenorhabditis elegans par21/mtssb-1 is essential for mitochondrial DNA replication and its defects causes comprehensive transcriptional alterations including a hypoxia response. *Experimental Cell Research, 314,* 103-114.

Szuhai, K.S., van den Ouweland, J.M., Dirks, R.W. et al. (2001). Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quantification in Myoclonus Epilepsy and Ragged-Red Fibers (MERRF) syndrome by multiplex Molecular Beacon based real-time fluorescence PCR. *Nucleic Acids Research, 29,* e13.

Waldbaum, S., and Patel, M. (2010a). Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy. *Journal of Bioenergetics and Biomembranes, 42,* 449-455.

Waldbaum, S., and Patel, M. (2010b). Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Research, 88,* 23-45.

Wei, Y., Lee, C., Lee, H., et al. (2001). Increases of mitochondrial mass and mitochondrial genome in association with enhanced oxidative stress in human cells harboring 4,977 bp-deleted mitochondrial DNA. *Annals of New York Academy of Sciences, 928,* 97-112.

Wiseman, H., and Halliwell, B. (1995). Damage to DNA by reactive oxygen species and nitrogen species role in inflammatory disease and progression to cancer. *Biochemical Journal, 313,* 17-29.

Wong, M. (2007). Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia, 48,* 617-630.

Wong, M. (2009). Animal models of focal cortical dysplasia and tuberous sclerosis complex: recent progress towards clinical applications. *Epilepsia, 50 suppl.9,* 34-44.

Wosik, K., Ruffini, F., Almazan, G., Olivera, A., Nalbantoglu, J.L. et al. (2004). Resistance of human adult oligodendrocytes to AMPA/kainite receptor-mediated glutamate injury. *Brain, 127,* 2636-2648.

Zsurka, G., Hample, K.G., Jardel, C., et al. (2010). Severe epilepsy as the major symptom of new mutations in the mitochondrial tRNA ^{Phe} gene. *Neurology, 74,* 507-512.

Table 1.1.
Clinical profile of pediatric patients with intractable epilepsy

Sample	Age (years)	Gender	Diagnosis	MCD Status	Pathology
7	15	F	intractable epilepsy	MCD	cortical dysplasia, type IA
9	18	F	intractable epilepsy	MCD	focal cortical dysplasia, Taylor type IIB
10	6	M	intractable epilepsy	Non-MCD	mild neuronal disorganization
11	13	F	intractable epilepsy	Non-MCD	cystic encephalomalacia
18	3	F	intractable epilepsy	Non-MCD	meningioangiomatosis
20	2	M	intractable epilepsy	Non-MCD	gliosis
23	7	F	intractable epilepsy	MCD	cortical dysplasia with Rasmussen's encephalitis
24	4	M	intractable epilepsy	MCD	cortical dysplasia, type IIA
25	4	F	intractable epilepsy	MCD	tuberous sclerosis
27	16	F	intractable epilepsy	MCD	cortical dysplasia, type IIB
30	5	M	intractable epilepsy	MCD	glioneuronal neoplasm/possible ganglioglioma
31	N/A	N/A	intractable epilepsy	Non-MCD	Information not available
33	N/A	N/A	intractable epilepsy	Non-MCD	Information not available
34	3	M	intractable epilepsy	MCD	cortical dysplasia
37	2	F	intractable epilepsy	MCD	cortical dysplasia with Rasmussen's encephalitis
38	17	F	intractable epilepsy	Non-MCD	Information not available
40	10	F	intractable epilepsy	MCD	cortical dysplasia, Palmini type IA
42	3	F	intractable epilepsy	MCD	cortical dysplasia, type 1A
43	9	M	intractable epilepsy	MCD	cortical dysplasia, type 1A
45	N/A	N/A	intractable epilepsy	Non-MCD	Information not available
48	6	M	intractable epilepsy	MCD	cortical dysplasia, Palmini type 1B

Table 1.2
Profile of control samples obtained from NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland

Sample	Age (years)	Gender	History/ Cause of Death
Control 1	15	F	car accident, multiple injuries
Control 2	18	F	car accident, multiple injuries
Control 3	13	F	asphyxia by hanging
Control 4	2	F	drowning
Control 5	2	F	drowning
Control 6	8	F	asphyxia and multiple injuries
Control 7	4	F	lymphocytic myocarditis
Control 8	16	F	car accident, multiple injuries
Control 9	2	F	car accident, multiple injuries
Control 10	17	F	car accident, multiple injuries
Control 11	10	F	Asthma

Table 1.3.
Mitochondrial SNPs and haplogroups used in this study

Variant	Region	Reported in Patients
A1555G	12S rRNA	Maternally inherited deafness or aminoglycoside-induced deafness
G3196A	16S rRNA	Alzheimer's Disease and Parkinson's Disease (ADPD)
T3197C	16S rRNA	Haplogroup (Hg)-U5
G4309A	tRNA Ile	Chronic Progressive External Ophthalmoplegia (CPEO)
A4317G	tRNA Ile	Fatal Infantile Cardiomyopathy (FICP)
G9804A	CO III	Leber Hereditary Optic Neuropathy (LHON)
G9952A	CO III	Mitochondrial Encephalopathy
A10006G	tRNA Gly	Chronic intestinal pseudo-obstruction (CIPO)
T10010C	tRNA Gly	Progressive encephalopathy (PEM)
A10398G	ND3	↓PD, ↓AD;A-↑Breast Cancer (BRCA) in AA, Hg-I, J, L, M

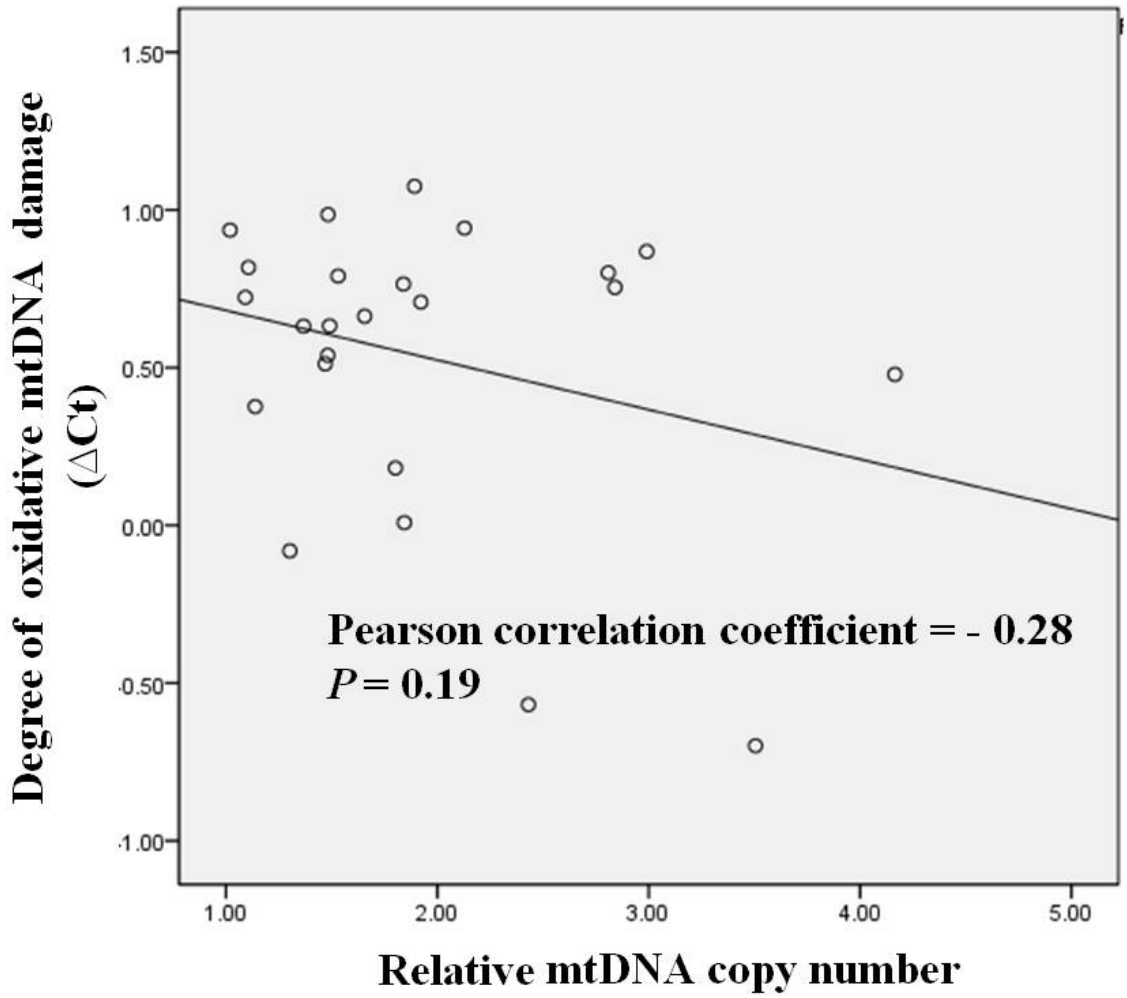

Figure 1.1.

Correlation between the mtDNA copy number and degree of oxidative mtDNA damage. The relative mtDNA copy numbers of the intractable epilepsy tissues and control tissues with their relationship to degree of oxidative mtDNA damage are plotted. The results show that the two parameters were not significantly correlated (Pearson correlation coefficient= -0.28, p= 0.19).

Table 1.4.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=29)	Non-neoplastic epilepsy (n=18)	Control (n=11)	*P*-value
Age (years)	8.13 ± 5.89	6.96 ± 5.37	9.73 ± 6.44	0.24
Sex (M/F)	7/19	7/8	0/11	0.01
log Relative mtDNA copy number				
Median	1.67	1.92	1.37	
Mean ± S.D.	1.87 ± 0.79	2.26 ± 0.84	1.33 ± 0.21	<0.001
> 1.67 (high) (%)		13 (87)	0 (0)	<0.001
≤ 1.67 (low) (%)		2 (13)	11 (100)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.66	0.74	0.63	
Mean ± S.D.	0.51 ± 0.49	0.43 ± 0.66	0.59 ± 0.27	0.47
> 0.66 (high damage) (%)		6 (40)	5 (45)	0.69
≤ 0.66 (low damage) (%)		9 (60)	6 (55)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.40	0.33	0.42	
Mean ± S.D.	0.36 ± 0.30	0.24 ± 0.31	0.46 ± 0.25	0.09
> 0.40 (high damage) (%)		4 (27)	6 (55)	0.66
≤ 0.40 (low damage) (%)		6 (60)	4 (36)	

Table 1. 5.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank by gender

	All Cases (n=29)	Epilepsy Male (n=7)	Epilepsy Female (n=8)	Control Female (n=11)	[a]*P*-value	[b]*P*-value	[c]*P*-value
Age (years)	8.13 ± 5.89	4.33 ± 2.91	9.25 ± 6.13	9.73 ± 6.44	0.70	0.03	0.87
log Relative mtDNA copy number							
Median	1.67	2.05	1.88	1.37			
Mean ± S.D.	1.87 ± 0.79	2.45 ± 1.02	2.10 ± 0.67	1.33 ± 0.21	0.44	0.03	0.01
> 1.67 (high) (%)		7 (100)	2 (25)	0 (0)	0.007	<0.001	0.001
≤ 1.67 (low) (%)		0 (0)	6 (75)	11 (100)			
log Degree of oxidative mtDNA damage (ΔCt)							
Median	0.66	0.90	0.36	0.63			
Mean ± S.D.	0.51 ± 0.49	0.77 ± 0.40	0.20 ± 0.72	0.59 ± 0.27	0.20	0.35	0.24
> 0.66 (high damage) (%)		3 (43)	3 (38)	5 (45)	0.57	0.56	1.00
≤ 0.66 (low damage) (%)		1 (14)	3 (38)	6 (55)			
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number							
Median	0.40	0.37	0.19	0.42			
Mean ± S.D.	0.36 ± 0.30	0.35 ± 0.20	0.17 ± 0.37	0.46 ± 0.25	0.40	0.44	0.07
> 0.40 (high damage) (%)		2 (29)	2 (25)	6 (55)	1.00	1.00	0.61
≤ 0.40 (low damage) (%)		2 (29)	4 (50)	4 (36)			

[a]P-value for Epilepsy, Male vs. Epilepsy, Female
[b]P-value for Epilepsy, Male vs. Control
[c]P-value for Epilepsy, Female vs. Control

Table 1.6.
Comparison of demographic and mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=23)	MCD (n=12)	Control (n=11)	*P*-value
Age (years)	8.10 ± 5.83	6.61 ± 5.02	9.73 ± 6.44	0.21
Sex (M/F)	5/18	5/7	0/11	0.04
log Relative mtDNA copy number				
Median	1.53	2.03	1.37	
Mean ± S.D.	1.86 ± 0.85	2.34 ± 0.93	1.33 ± 0.21	0.002
> 1.53 (high) (%)		10 (83)	1 (9)	0.002
≤ 1.53 (low) (%)		2 (17)	9 (82)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.63	0.44	0.63	
Mean ± S.D.	0.47 ± 0.49	0.30 ± 0.68	0.59 ± 0.27	0.28
> 0.63 (high damage) (%)		4 (33)	5 (45)	1.00
≤ 0.63 (low damage) (%)		4 (33)	4 (36)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.36	0.20	0.42	
Mean ± S.D.	0.34 ± 0.31	0.18 ± 0.32	0.46 ± 0.25	0.05
> 0.36 (high damage) (%)		2 (17)	7 (64)	0.15
≤ 0.36 (low damage) (%)		5 (42)	3 (27)	

Table 1.7.
Comparison of demographic and mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=17)	Non-MCD (n=6)	Control (n=11)	*P*-value
Age (years)	8.33 ± 7.77	6.96 ± 5.37	9.72 ± 6.44	0.24
Sex (M/F)	2/12	2/1	0/11	0.03
log Relative mtDNA copy number				
Median	1.49	2.43	1.37	
Mean ± S.D.	1.78 ± 0.82	2.60 ± 0.88	1.33 ± 0.21	0.02
> 1.49 (high) (%)		6 (100)	8 (73)	0.01
≤ 1.49 (low) (%)		0 (0)	2 (18)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.69	0.77	0.63	
Mean ± S.D.	0.65 ± 0.26	0.77 ± 0.21	0.59 ± 0.27	0.21
> 0.69 (high damage) (%)		4 (67)	4 (36)	0.28
≤ 0.69 (low damage) (%)		1 (17)	7 (64)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.41	0.28	0.42	
Mean ± S.D.	0.42 ± 0.23	0.24 ± 0.31	0.46 ± 0.25	0.31
> 0.41 (high damage) (%)		1 (17)	6 (55)	0.57
≤ 0.41 (low damage) (%)		3 (50)	5 (45)	

Table 1.8.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-MCD

	All Cases (n=18)	MCD (n=12)	Non-MCD (n= 6)	*P*-value
Age (years)	6.96 ± 5.37	6.6 ± 5. 01	8.33 ± 7.78	0.63
Sex (M/F)	2/12	2/1	5/12	0.57
log Relative mtDNA copy number				
Median	2.09	2.03	2.43	
Mean ± S.D.	2.43 ± 0.89	2.34 ± 0.93	2.60 ± 0.89	0.58
> 2.09 (high) (%)		6 (50)	3 (50)	1.00
≤ 2.09 (low) (%)		6 (50)	3 (50)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.75	0.44	0.76	
Mean ± S.D.	0.48 ± 0.58	0.30 ± 0.68	077. ± 0.21	0.10
> 0.75 (high damage) (%)		3 (25)	3 (50)	0.55
≤ 0.75 (low damage) (%)		5 (42)	1 (17)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.29	0.20	0.29	
Mean ± S.D.	0.24 ± 0.27	0.18 ± 0.32	0.33 ± 0.17	0.36
> 0.29 (high damage) (%)		3 (25)	2 (33)	1.00
≤ 0.29 (low damage) (%)		4(12)	3 (50)	

Table 1.9.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Epilepsy (n=18)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	15	83	11	100	0.27	0	0, NaN	0	0, NaN
G3196A	7	39	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	15	83	11	100	0.27	0	0, NaN	0	0, NaN
A4317G	15	83	11	100	0.27	0	0, NaN	0	0, NaN
G9804A	2	11	0	0	0.51	1.11E9	0, NaN	1.27E9	0, NaN
G9952A	3	17	0	0	0.27	1.19E9	0, NaN	1.48E9	0, NaN
A10006G	1	5	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	5	28	7	64	0.12	0.22	0.04, 1.09	0.29	0.05, 1.55
A10398G	1	5	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.10.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Epilepsy (n=18)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	1	5	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	1	5	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	1	5	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	1	5	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.11.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy male patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Epilepsy Male (n=7)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	6	86	11	100	0.39	0	0, NaN	0	0, NaN
G3196A	3	43	11	100	0.01	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	6	86	11	100	0.39	0	0, NaN	0	0, NaN
A4317G	5	71	11	100	0.14	0	0, NaN	0	0, NaN
G9804A	1	14	0	0	0.39	2.96E9	0, NaN	1.39E9	0, NaN
G9952A	2	29	0	0	0.14	3.55E9	0, NaN	2.17E9	0, NaN
A10006G	1	14	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	3	43	7	64	0.63	0.43	0.06, 2.97	0.48	0.05, 4.29
A10398G	0	0	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table1. 12.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy male patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Epilepsy Male (n=7)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.13.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy female patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Epilepsy Female (n=8)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	6	75	11	100	0.16	0	0, NaN	0	0, NaN
G3196A	2	25	11	100	0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.001	0	0, NaN	0	0, NaN
G4309A	6	75	11	100	0.16	0	0, NaN	0	0, NaN
A4317G	7	88	11	100	0.42	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	NaN	NaN, NaN	1.01	0.87, 1.18
G9952A	1	13	0	0	0.42	2.54E9	0, NaN	3.15E9	0, NaN
A10006G	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	2	25	7	64	0.17	0.14	0.02, 1.16	0.19	0.25, 1.45
A10398G	1	13	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1. 14.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy female patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Epilepsy Female (n=8)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	1	13	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	1	13	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	1	13	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	1	13	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.15.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	MCD (n=12)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	10	83	11	100	0.48	0	0, NaN	0	0, NaN
G3196A	5	42	11	100	0.01	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	9	75	11	100	0.22	0	0, NaN	0	0, NaN
A4317G	9	75	11	0	0.22	0	0, NaN	0	0, NaN
G9804A	1	8	0	0	1.00	1.62E9	0, NaN	1.01E9	0, NaN
G9952A	1	8	0	0	1.00	1.62E9	0, NaN	4.27E9	0, NaN
A10006G	0	0	11	100	<0.001	0	0, NaN	---	---
T10010C	3	25	7	64	0.10	0.19	0.03, 1.14	0.22	0.04, 1.38
A10398G	1	8	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.16.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	MCD (n=12)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	1	8	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	1	8	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	1	8	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	1	8	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.17.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Non-MCD (n=6)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	5	83	11	100	0.35	0	0, NaN	0	0, NaN
G3196A	2	33	11	100	0.01	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	6	100	11	100	1.00	NaN	0, NaN	NaN	0, NaN
A4317G	6	100	11	100	1.00	NaN	0, NaN	NaN	0, NaN
G9804A	1	17	0	0	0.35	3.55E9	0, NaN	NaN	0, NaN
G9952A	2	33	0	0	0.11	4.44E9	0, NaN	1.78E10	0, NaN
A10006G	1	17	11	100	<0.001	0	0, NaN	7.30E13	0, NaN
T10010C	2	33	7	64	0.34	0.29	0.04, 2.32	1.07	0.07, 16.39
A10398G	0	0	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.18.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Non-MCD (n=6)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.19.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-MCD

SNPs	MCD (n=12)		Non-MCD (n=6)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	10	83	5	82	1.00	1.00	0.07, 13.87	0.37	0.02, 6.69
G3196A	5	42	2	30	1.00	1.43	0.18, 11.09	0	0, NaN
T3197C	0	0	0	0	1.00	NaN	NaN, NaN	0.94	0.75, 1.19
G4309A	9	75	6	100	0.52	0	0, NaN	0	0, NaN
A4317G	9	75	6	100	0.52	0	0, NaN	0	0, NaN
G9804A	1	8	1	17	1.00	0.45	0.02, 8.83	0	0, NaN
G9952A	1	8	2	33	0.25	0.18	0.01,2.60	0.05	0.90, 548.77
A10006G	0	0	1	17	0.33	0	0, NaN	0	0, NaN
T10010C	3	25	2	33	1.00	0.67	0.08, 5.68	0.15	0.39, 111.37
A10398G	1	8	0	0	1.00	0	0, NaN	0	0, NaN

[a]OR, Crude Odds ratio using non-MCD as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.20.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-MCD

Haplogroups	MCD (n=12)		Non-MCD (n=6)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	1	8	0	0	1.00	0	0, NaN	0	0, NaN
Hg-J	1	8	0	0	1.00	0	0, NaN	0	0, NaN
Hg-L	1	8	0	0	1.00	0	0, NaN	0	0, NaN
Hg-M	1	8	0	0	1.00	0	0, NaN	0	0, NaN
Hg-U5	0	0	0	0	1.00	NaN	NaN, NaN	0.94	0.75, 1.19

[a]OR, Crude Odds ratio using non-MCD as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 1.21.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0.32 (0, NaN)	2.35E17 (0, NaN)	2.34E8 (0, NaN)	1.15 (0.72, 1.84)
G3196A	---	---	---	---
T3197C	0 (0, NaN)	9.69E27 (0, NaN)	1.02E23 (0, NaN)	33.39 (0, NaN)
G4309A	0.12 (0, NaN)	1.49E17 (0, NaN)	2.39E8 (0, NaN)	1.52 (0.72, 1.84)
A4317G	1.00E8 (0, NaN)	4.40E17 (0, NaN)	2.66E8 (0, NaN)	1.15 (0.72, 1.84)
G9804A	0 (0, NaN)	4.44E17 (0, NaN)	2.67E8 (0, NaN)	1.15 (0.72, 1.84)
G9952A	0 (0, NaN)	4.40E17 (0, NaN)	2.66E8 (0, NaN)	1.15 (0.72, 1.84)
A10006G	---	---	---	---
T10010C	1.69E38 (0, NaN)	8.32E90 (0, NaN)	8.56E37 (0, NaN)	4.24E4 (0, NaN)
A10398G	---	---	---	---
Hg-I	---	---	---	---
Hg-J	---	---	---	---
Hg-L	---	---	---	---
Hg-M	---	---	---	---
Hg-U5	0 (0, NaN)	9.69E27 (0, NaN)	1.02E23 (0, NaN)	33.39 (0, NaN)

OR, using control group as reference
NaN = Not a number

Table 1.22.
The results of the logistic regression for SNPs/Hg, level of index of level relative mtDNA copy number by level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric intractable epilepsy male patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of Index OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	0.31 (0.02, 5.93)	1.23 (0.87, 1.74)
G3196A	0 (0, NaN)	0 (0, NaN)	1.12 (0.74, 1.69)
T3197C	0 (0, NaN)	0 (0, NaN)	1.18 (0.77, 1.81)
G4309A	---	0.68 (0.05,8.64)	1.21 (0.91, 1.60)
A4317G	0 (0, NaN)	1.52 (0.08, 29.20)	1.23 (0.88, 1.72)
G9804A	4.85E9 (0, NaN)	0.366 (0.02, 6.15)	1.16 (0.88, 1.54)
G9952A	8.89E9 (0, NaN)	0.31 (0.02, 5.93)	1.23 (0.87, 1.74)
A10006G	---	---	---
T10010C	0.89 (0.07, 11.83)	0.68 (0.05, 8.72)	1.20 (0.91, 1.60)
A10398G	---	---	---
Hg-I	---	---	---
Hg-J	---	---	---
Hg-L	---	---	---
Hg-M	---	---	---
Hg-U5	0 (0, NaN)	0 (0, NaN)	1.18 (0.77, 1.81)

OR, using control group as reference
Nan, not a number

Table 1.23.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric intractable epilepsy female patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0.15 (0, NaN)	4.02E17 (0, NaN)	3.55E8 (0, NaN)	1.15 (0.72, 1.84)
G3196A	---	---	---	---
T3197C	0 (0, NaN)	4.84E28 (0, NaN)	2.59E23 (0, NaN)	35.33 (0, NaN)
G4309A	0.13 (0, NaN)	2.35E17 (0, NaN)	3.57E8 (0, NaN)	1.15 (0.72, 1.84)
A4317G	---	7.94E17 (0, NaN)	3.54E8 (0, NaN)	1.15 (0.72, 1.84)
G9804A	---	7.94E17 (0, NaN)	3.54E8 (0, NaN)	1.15 (0.72, 1.84)
G9952A	---	7.94E17 (0, NaN)	3.54E8 (0, NaN)	1.15 (0.72, 1.84)
A10006G	---	---	---	---
T10010C	2.47E39 (0, NaN)	6.47E93 (0, NaN)	1.14E39 (0, NaN)	6.0E4 (0, NaN)
A10398G	---	---	---	---
Hg-I	---	---	---	---
Hg-J	---	---	---	---
Hg-L	---	---	---	---
Hg-M	---	---	---	---
Hg-U5	0 (0, NaN)	4.84E28 (0, NaN)	2.59E23 (0, NaN)	35.33 (0, NaN)

OR, using control group as reference
NaN = Not a number

Table 1.24.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	35.55 (1.73, 732.75)	1.57 (0.06, 42.17)	1.10 (0.82, 1.50)
G3196A	0 (0, NaN)	5.60E42 (0, NaN)	12.61 (0, NaN)	9.29E6 (0, NaN)
T3197C	0 (0, NaN)	1.18E14 (1.40, 620.98)	0 (0, NaN)	38.68 (0, NaN)
G4309A	0 (0, NaN)	24.39 (1.40, 620.98)	1.95 (0.07, 55.61)	1.10 (0.81, 1.48)
A4317G	0 (0, NaN)	38.29 (1.89, 777.18)	0.99 (0.05, 21.77)	1.08 (0.81, 1.43)
G9804A	1.99E8 (0, NaN)	39.24 (1.94, 792.85)	1.00 (0.45, 22.36)	1.07 (0.80, 1.42)
G9952A	---	44.31 (2.22, 886.30)	1.15 (0.05, 24.90)	1.08 (0.81, 1.45)
A10006G	---	---	---	---
T10010C	0.42 (0.02, 9.62)	37.07 (1.78, 771.30)	1.22 (0.06, 26.23)	1.07 (0.80, 1.43)
A10398G	---	---	---	---
Hg-I	---	---	---	---
Hg-J	---	---	---	---
Hg-L	---	---	---	---
Hg-M	---	---	---	---
Hg-U5	0 (0, NaN)	1.18E14 (1.40, 620.98)	0 (0, NaN)	38.68 (0, NaN)

OR, using control group as reference
NaN = Not a number

Table 1.25.
The results of the logistic regression for haplogroups, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	1.54E16 (0, NaN)	1140 (0, NaN)	0 (0, NaN)
G3196A	---	---	---	---
T3197C	---	---	---	---
G4309A	---	1.20E9 (0, NaN)	5.00E8 (0, NaN))	1.05 (0.62, 1.87)
A4317G	---	1.28E9 (0, NaN)	5.00E8 (0, NaN))	1.05 (0.62, 1.87)
G9804A	---	1.20E9 (0, NaN)	5.00E8 (0, NaN))	1.08 (0.62, 1.87)
G9952A	7.86E42 (0, NaN)	1.54E16 (0, NaN)	1140 (0, NaN)	0 (0, NaN)
A10006G	---	---	---	---
T10010C	0.0 (0, NaN)	4.42E48 (0, NaN)	2.56E11 (0, NaN)	151.07 (0, NaN)
A10398G	---	---	---	---
Hg-I	---	---	---	---
Hg-J	---	---	---	---
Hg-L	---	---	---	---
Hg-M	---	---	---	---
Hg-U5	0 (0, NaN)	1.18E14 (1.40, 620.98)	0 (0, NaN)	38.68 (0, NaN)

OR, using control group as reference
NaN = Not a number

Table 1.26.
The results of the logistic regression for haplogroups, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-MCD

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	---	---	---	---
G3196A	0.46 (0, NaN)	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
T3197C	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
G4309A	0.30 (0, NaN)	0 (0, NaN)	5.61E9 (0, NaN)	0.76 (0.32, 1.84)
A4317G	10.07 (0, NaN)	0 (0, NaN)	4.17E9 (0, NaN)	0.76 (0.32, 1.84)
G9804A	0.49 (0, NaN)	0 (0, NaN)	4.03E9 (0, NaN)	0.76 (0.32, 1.84)
G9952A	---	---	---	---
A10006G	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
T10010C	0 (0,NaN)	0 (0, NaN)	1.31E117 (0, NaN)	2.50E14 (0, NaN)
A10398G	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
Hg-I	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
Hg-J	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
Hg-L	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
Hg-M	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)
Hg-U5	---	0 (0, NaN)	3.99E9 (0, NaN)	0.76 (0.32, 1.84)

OR, using Non-MCD as reference
NaN = Not a number

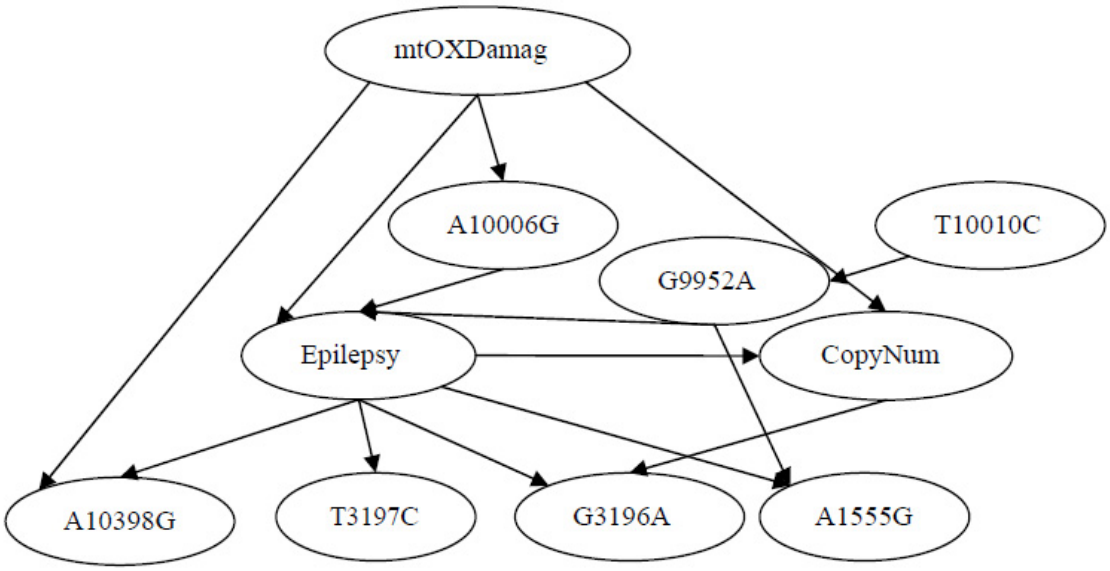

Figure 1.2.

Summary Beyesian network based on the top three Bayesian networks for female samples. Absolute value on influence score higher than 0.1 are shown.

Table 1.27.
Influence scores between selected variables for female samples

Variables	Influence Score
(Epilepsy) -> (T3197C)	0.7826
(Epilepsy) -> (G3196A)	0.6288
(Epilepsy) -> (A10398G)	0.5383
(A10006G) -> (Epilepsy)	0.4858
(G9952A) -> (Epilepsy)	-0.4475
(Epilepsy) -> (A1555G)	0.1251

Table 1.28.
Pearson correlation between selected variables based on the influence scores for female samples

	T3197C	G3196A	A10006G	A10398G	G9952A	mcd status
Epilepsy	-.832**	-.832**	-.832**	-.913**	.471*	-.896**
Sig.	0	0	0	0	0.027	0
mcd status	.746**	.817**	.556**	.853**	-.573**	1
Sig.	0	0	0.007	0	0.005	
mtOXDamageDbyA10398G	0.179	0.009	0.383	0.235	0.141	0.088
Sig.	0.426	0.97	0.079	0.292	0.531	0.696
mtOXDamageDbyCopyNum	-.535*	-0.383	-.467*	-.705**	0.3	-.542**
Sig.	0.01	0.078	0.028	0	0.175	0.009
mtOXDamageD	-0.203	-0.354	0.017	-0.267	0.28	-0.339
Sig.	0.366	0.106	0.941	0.23	0.207	0.123
mtOXDamage	-0.189	-0.373	0.213	-0.246	.575**	-0.349
Sig.	0.399	0.087	0.342	0.27	0.005	0.112
CopyNum	-.477*	-0.189	-.523*	-.628**	0.154	-0.409
Sig.	0.025	0.399	0.013	0.002	0.492	0.059
copyno	-0.221	0.15	-0.317	-0.293	-0.106	-0.145
Sig.	0.323	0.505	0.15	0.186	0.639	0.52

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

Table 1.28. (cont.)
Pearson correlation between selected variables based on the influence scores for female samples

		mtOXDamageDbyA10398G	mtOXDamageDbyCopyNum	mtOXDamageD	mtOXDamage	CopyNum	copyno
Epilepsy		-0.276	.616**	0.183	0.137	.573**	0.268
	Sig.	0.213	0.002	0.416	0.543	0.005	0.228
mcd status		0.088	-.542**	-0.339	-0.349	-0.409	-0.145
	Sig.	0.696	0.009	0.123	0.112	0.059	0.52
mtOXDamageDbyA10398G		1	0.106	.874**	.648**	-0.347	-0.367
	Sig.		0.639	0	0.001	0.114	0.093
mtOXDamageDbyCopyNum		0.106	1	.457*	0.351	.865**	.432*
	Sig.	0.639		0.032	0.11	0	0.045
mtOXDamageD		.874**	.457*	1	.765**	-0.03	-0.217
	Sig.	0	0.032		0	0.895	0.331
mtOXDamage		.648**	0.351	.765**	1	-0.036	-0.253
	Sig.	0.001	0.11	0		0.872	0.257
CopyNum		-0.347	.865**	-0.03	-0.036	1	.715**
	Sig.	0.114	0	0.895	0.872		0
copyno		-0.367	.432*	-0.217	-0.253	.715**	1
	Sig.	0.093	0.045	0.331	0.257	0	

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

CHAPTER IV

MANUSCRIPT 2

MITOCHONDRIAL DNA BACKGROUND AND OXIDATIVE DAMAGE IN INTRACTABLE EPILEPSY

PEDIATRIC PATIENTS WITH BRAIN TUMORS

Abstract

Objectives: Research mitochondrial background and mitochondrial DNA (mtDNA) damage in neoplastic pediatric intractable epilepsy patients. mtDNA oxidative damage and copy number are indices of mitochondrial damage. Mitochondrial damage may play a role in the pathology of intractable epilepsy. The purpose of this study is to determine and compare mtDNA variants (SNPs) and mtDNA oxidative damage in neoplastic pediatric intractable epilepsy patients with malformations of cortical development (MCD) and non-MCD with non-epileptic controls.

Methods: Brain tissue specimens were collected from 27 neoplastic pediatric intractable epilepsy patients from Miami Children's Hospital and 11 controls (non-epileptic) from UMB. Oxidative mtDNA damage as indicated by $mtDNA^{\Delta Ct}$ (formation of 8-OHdG) and relative mtDNA copy number were determined for each tissue by quantitative real-time PCR (QPCR). A total of 10 SNPs associated with mitochondrial myopathies were genotyped by allele-specific oligonucleotide dot (ASO) blot analysis. In female samples, a summary Bayesian network was created to investigate the relationship of these variables.

Results: Relative mtDNA copy number were higher in female neoplastic intractable epilepsy patients compared to non-epileptic control samples (p=0.34). Oxidative mtDNA damage was higher in female neoplastic epileptic compared to control samples (p=0.72). mtSNP G9952A was found in higher frequencies in female neoplastic epilepsy samples. Female neoplastic epilepsy phenotypes were predicted by G9952A, level of relative mtDNA copy number, and level of oxidative mtDNA damage. Bayesian network showed relationships ($p < 0.05$) between brain tumor with G9952A, A1555G, T3197C, A10006G, A10398G, oxidative mtDNA damage with relative mtDNA copy number, and brain tumor with relative mtDNA copy number in female samples.

Conclusion: These data suggest that the mtSNPs explored are associated with neoplastic intractable epilepsy phenotypes. mtDNA copy number and $mtDNA^{\Delta Ct}$ were higher in neoplastic epilepsy samples but did not reach statistical significance. Studies exploring mitochondrial compensation in response to oxidative damage in neoplastic epilepsy are needed.

Epilepsy is a chronic neurological disorder characterized by spontaneous recurring seizures. The incidence of epilepsy is highest for children and the elderly. A proportion of patients have seizures that are resistant to traditional anti-epilepsy medicines (intractable or refractory epilepsy) (Alexander and Godwin, 2006). Brain tumors are a common cause of epilepsy (Govori et al., 2010). The occurrence of epilepsy is highly associated with malformations of cortical development (MCD), which are developmental brain lesions that consist of dysplastic neuronal lesions (malformations) (Schwartzkroin and Walsh, 2000; Hua and Crino, 2003; Hader et al., 2004; Rickert, 2006; Wong, 2007). MCD are increasingly being recognized as the cause of intractable epilepsy. MCD presents a broad spectrum of structural changes which appear to result from changes in precursor neuronal or neuronal cells during cortical development at various stages such as: proliferation, migration, differentiation, and apoptosis (Becker et al., 2004). MCDs include gangliomas and dysembryoplastic neuroepithelial tumors (DNETs) (Majores et al., 2007). Gangliogliomas are composed of neoplastic, highly differentiated glial cells, and dysplastic neurons (Schick et al., 2007). DNETs are benign lesions of the cerebral cortex that are composed of glial and oligodendrocyte-like cells (neuronal elements) which resemble gliomas (Spalice et al., 2010).

Treatment with anti-epileptic drugs (AEDs) is usually ineffective, and children with MCD may require surgical removal of the affected area of the brain (Yasin et al., 2010). In surgical series, focal cortical malformations and low-grade tumors were the most common in infants with intractable epilepsy. Gangliogliomas account for 5% of childhood tumors (Schick et al., 2007a). According to Saneto and Wyllie (2000), low-grade tumors included gangliomas, gangliocytomas, DNETs, and astrocytomas. DNETs

are often present with epilepsy during childhood (Chang et al., 2010). Gangliogliomas are the most frequent tumors found in patients with focal epilepsy (Schick et al., 2007). The molecular mechanisms underlying the formation of MCD are still largely unknown and the treatments for epilepsy due to MCD are often ineffective or limited (Wong, 2009).

Through the mutation and dysregulation of critical genes, oxidative DNA damage has been implicated in the development of several human cancers (Kim et al., 2004). Oxidative insults may cause molecular damage that can drive the progression of normal tissue to cancer. Oxidative DNA damage has been detected in cancer and neurodegenerative diseases (Tsutsui et al., 2001). Alterations of mitochondria have been found in the human cancers including breast, esophageal, gastric, non-small-cell-lung cancer, and thyroid cancers (Kim et al., 2004). Mitochondria contain their own genome, mtDNA, which consists of a 16.5-kb circular double-stranded DNA (dsDNA) molecule containing 37 genes (Bai et al., 2008). Mitochondria functions in oxidative phosphorylation, heme, lipid, amino acid biosynthesis, and fatty acid oxidation among other functions (Sugimoto, 2008). The major source of ATP in cortical neurons is provided by mitochondrial oxidative phosphorylation (Chuang, 2010; Waldbaum and Patel, 2010a). Mammalian cells contain several hundreds to more than a thousand mitochondria. The size, shape and abundance of mitochondria vary drastically in different cell types and may change under differing energy demands and physiological or environmental conditions. In a cell, the abundance of mitochondria is determined by biogenesis and division of the organelles. The quantity of mitochondria per cell is tighly

regulated by activation of specific transcription factors and signaling pathways (Lee and Wei, 2005).

Mitochondrial metabolism also generates the majority of the reactive oxygen species (ROS) production in cells (St-Pierre et al., 2006). ROS results when unpaired electrons escape the electron transport chain. The most commonly produced base lesion by ROS, and the most often measured as an index of oxidative DNA damage is 8-hydroxyguanosine (8-OHdG) (Wiseman and Halliwell, 1995). The brain is believed to be particularly susceptible to the damaging effects of reactive oxygen species (ROS) damage due to its high metabolic rate and reduced capability for cellular regeneration compared to other organs (Anderson, 2004). ROS has been implicated in the initial phases of seizure-induced pathology and several studies have reported oxidative stress in different regions of the brain following experimental seizures (Devi et al., 2008).

Mitochondrial oxidative stress and dysfunction are contributing factors to several neurological disorders (Shokolenko et al., 2009; Waldbaum and Patel, 2010b). Encephalomyopathies have been found in children in association with defects in mitochondrial structure and function. Some of these disorders are acute leukemia, Alzheimer's disease (AD), cardiomyopathy (CM), chronic progressive external ophthalmoplegia, colorectal cancer, deafness, fatal infantile cardiomyopathy (FICP), gastroesopageal reflux (GER), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), Leber Hereditary Optic Neuropathy (LHON), Parkinson's disease (PD), maternally inherited hypertropic cardiomyopathy (MHCM), multiple sclerosis (MS), non-insulin dependent diabetes mellitus (NIDDM), non-syndromic hearing loss, progressive

encephalopathy (PEM), sensorineural hearing loss (SNHL), strokes, and sudden infant death syndrome (SIDS) (Filiano et al., 2002; Bai et al., 2007; Tzen et al., 2007). In this study, we investigate the presence of oxidative mtDNA damage, constitutional mtDNA copy number, mtDNA SNPs, haplogroups as sources of genetic diversity that may contribute to the development of neoplastic intractable epilepsy.

Methods

Collection of brain tissue from pediatric intractable epilepsy patients

Brain tissue samples from 25 pediatric neoplastic intractable epilepsy patients who have undergone brain resection surgery at the Miami Children's Hospital, Miami, FL, during 2008-2009 were collected. Tissues obtained during surgical resection were immediately snap-frozen in liquid nitrogen and stored at -80°C. As references, eleven normal, non-epileptic, pediatric brain tissues were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD and stored at -80°C. This study was approved by the Florida International University's Institutional Review Board.

DNA extraction

Freshly excised human neuronal (brain) tissue was stored in liquid nitrogen and frozen in -80°C until ready for processing. The frozen neuronal tissue was homogenized, while on ice, using a Janke and Kunkle TP-18-10 blade type homogenizer in which 1 ml of Trizol® was added. The homogenate was transferred to a 2.0-ml microcentrifuge tube. DNA was then isolated and purified via Phenol-Chloroform extraction. The precipitated DNA was pelleted and resuspended in1X TE buffer. The integrity of the DNA was verified following electrophoresis through 2% agarose gels.

Determination of and mtDNA copy number and mitochondrial DNA oxidative damage by quantitative real time PCR

Quantitative real-time PCR (QPCR) assays were performed using Applied Biosystems 7300 System with a final volume of 25uL reaction mixture containing 50ng DNA template, 12.5uL SYBR Green PCR Master Mix (Qiagen), and 10mM of each primer. The sequences for the primers used for the amplification of the mitochondrial gene *NADH Dehydrogenase 1* (*ND1)* were: mtF3212, 5'-CACCCAAGAACAGGGTTTGT-3' and mtR3319, 5'-TGGCCATGGGATTGTTAA-3'. The sequences of the primer for the nuclear housekeeping gene *18s rRNA*, used for the normalization in the QPCR analysis, were: 18s1546F, 5'-TAGAGGGACAAGTGGCGTTC-3'and 18s650R, 5'-CGCTGAGCCAGTCAGTGT-3' (Bai et al., 2004; Lin et al, 2008). The QPCR conditions were set up as follows: hot start at 95° C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The fluorescence intensity was measured at the end of the extension phase at 60°C. All samples were performed in duplicated and a non-template negative control was included in each reaction.

A total of 50ng of DNA was used in QPCR for the determination of the threshold cycle number (Ct) of the *18s rRNA* and *ND1* genes. Ct values can be used as a measure of input copy number and the Ct value differences were used to quantify the mtDNA copy number relative to the 18s rRNA gene with the following equation:
Relative copy number= $2^{\Delta Ct}$, where ΔCt is the Ct *18s rRNA* – Ct *ND1* (Szuhai et al., 2001; Bai et al., 2004; Lewis et al., 2007; Edwards, 2009; Lee et al, 2010).

Since degree of oxidative mtDNA damage is reflected by an abundance of 8-OHdG formation in mtDNA, the content of 8-OHdG in mtDNA, an index for cellular oxidative damage, was determined by QPCR and presented as ΔCt (Ayala-Torres et al., 2000). As indicated by Lin et al (2008), the amplification efficiency would decrease after treatment of the DNA sample with hOGG1 to remove the 8-OHdG residue to form an abasic site. The content of 8-OHdG in the samples' mtDNA was determined by treating the 200ng sample with 2 units of hOGG1 at 37° C for 1 hour and 5 minutes to remove the 8-OHdG residue to form an basic site. The digested mtDNA were amplified by QPCR using the primers for *ND1* gene. PCR amplification efficiencies of DNA templates containing a single 8-OHdG or two 8-OHdGs at least 13 base pairs apart are not significantly disturbed (Ct_1), however, the presence of an abasic site in DNA after treatment of hOGG1 would dramatically reduce the PCR efficiency, thus, increasing the Ct value (Ct_2) (Lin et al., 2008). The degree of oxidative mtDNA damage, $mtDNA^{\Delta Ct}$, was defined as $\Delta Ct = Ct_2$ (hOGG1 treatment) – Ct_1 (no hOGG1 treatment) (Lin et al., 2008; Su et al., 2010). Each analysis was performed in duplicate, and the mean value of ΔCt was calculated for each sample. Hence, the larger the ΔCt, the more abundant the 8-OHdG and more oxidative damage present in the sample.

mtDNA genotyping

A total of 50ng of DNA was used in Quantitative PCR (QPCR) to amplify the mitochondrial genome with 19 primer sets (Table A.8) designed by Bai et al (2007) to include the mtDNA regions containing 10 reported mtDNA variations (SNPs). The mtDNA variations are distributed along the rRNA, tRNA, COIII and ND3 regions of the mitochondrial genome. The mtDNA variations studied have been reported in patients

with varying diseases such as: Alzheimer's disease, Parkinson's disease, Leber's hereditary optic neuropathy (LHON), deafness, chronic progressive external ophthalmoplegia (CPEO), chronic intestinal pseudo-obstruction (CIPO) and other diseases (Table 2.3).

QPCR assays to amplify 19 regions of the mitochondrial genome (Table A.8) were performed using Applied Biosystems 7300 System with a final volume of 25uL reaction mixture containing 50ng DNA template, 12.5uL SYBR Green PCR Master Mix (Qiagen), and 10mM of each primer. The QPCR conditions were set up as follows: hot start at 95° C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Two microliters of QPCR products were spotted on Hybond N+ membrane (GE). Dot blot preparation and hybridization conditions are available in the Appendix. The allele-specific oligonucleotide (ASO) probes for the mtDNA variants are listed in Table A.9 (Bai et al, 2007). These probes were labeled with Dioxigenin (DIG) (Roche, DIG Oligonucleotide 3'-End Labeling Kit). Both the variant and wild type controls for each ASO blot were included as controls. mtDNA haplogroups I, J, L, M and U5 were classified according to Table A.7.

Bayesian network

In female samples, Bayesian Network Inference with Java Objects (Banjo) © software was used to determine influence scores and to create a directed acyclic graph (DAG) to represent the Bayesian network showing the probablilistic relationships between epilepsy status, MCD status, relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs with influence scores (absolute value) higher than 0.1. The top three scoring networks were used to generate a consensus network.

Statistical Analysis

Continuous variables were studentized to identify and remove outliers. Logarithmic transformation of data was used since the original values of the relative mtDNA copy number and mtDNA oxidative damage showed non normal distributions. The continuous variables between groups were compared using the Student's t-test. Pearson's correlation test was used to test the relationship between mtDNA copy number and mtDNA oxidative damage. Analysis was carried out using Fisher's exact test for each individual SNP and haplogroup.

Logistic regression was used to determine the odds ratios. Neoplastic epilepsy patients were sub-divided as those being malformations of cortical development (MCD) and non-MCD as per Barkovich MCD classification system (Barkovich et al., 2005). Logistic regression was also used to explore if neoplastic epilepsy, MCD, or non-MCD phenotypes were dependent on mtSNPs, haplogroups, level of mtDNA oxidative damage, and level of relative mtDNA copy number, model adjusted by age. In female samples, Banjo © software was used to was used to determine influence scores and to create a directed acyclic graph (DAG) to represent the Bayesian network showing the probablilistic relationships between brain tumor status, MCD status relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs. In addition, Pearson correlations were determined for these relationships. Statistical analyses were performed using SPSS version 18.0 for Microsoft Windows. Statistical significance was set at $P < 0.05$.

Results

Demographics and clinical information

The demographic and pathology information of the 27 pediatric neoplastic intractable epilepsy patients (MCD = 6, non-MCD = 21) are shown in Table 2.1, and for the 11 control samples in Table 2.2. MCD cases accounted for 22% of the neoplastic intractable epilepsy cases. All of the epilepsy patients with MCD are classified as malformations due to abnormal glial and neuronal proliferation. The groups did not significantly differ in ages. Using correlation methods, we demonstrate that mtDNA copy number and the degree of oxidative DNA damage were not significantly correlated (Pearson correlation=0.14, p=0.45) (Fig. 2.1). However, in female samples the mtDNA copy number and the degree of oxidative DNA damage were significantly correlated (Pearson correlation=-0.61, p=0.001) (Table 2.28).

Relative mtDNA copy number

The measurement of DNA in individual amplifiable DNA segments is measured by quantitative real-time PCR (QPCR). The use of SYBR green in QPCR assays allows for the detection of small difference in the starting template using nanograms of DNA. The higher threshold cycle (Ct), or shift of the amplification curve to the right, indicates a low amount of starting DNA template, thus, decreased DNA content. Ratios of mtDNA/nuclear DNA were used to obtain the relative mtDNA copy number, where a lower ratio is representative of a lower initial DNA template, demonstrating a decrease in the amount of mtDNA. We selected the nuclear *18s rRNA* gene and mtDNA coding region of *ND1* gene. We used QPCR to analyze the relative mtDNA copy number of the neoplastic pediatric intractable epilepsy brain tissues and non-epileptic control brain

tissues. Tables 2.4 -2.8 show the average relative mtDNA copy number for each group. The relative mtDNA copy number (p=0.82) did not significantly differ between male and female neoplastic epilepsy patients (Table 2.5). A 21% increase in mtDNA copy number for neoplastic epilepsy samples was observed compared to controls (p=0.26). Both MCD (2.03, p=0.14) and non-MCD (1.48, p=0.59) samples demonstrated a trend of higher mtDNA copy numbers compared to controls (1.33). MCD patients had a higher relative mtDNA copy number compared to non-MCD patients, however, statistical significance was not reached (p=0.33). In addition, female neoplastic epilepsy samples presented a trend of higher mtDNA copy number than controls (p=0.34).

Oxidative mtDNA damage

The degree of oxidative mtDNA damage is reflected by an abundance of 8-OHdG formation in mtDNA, the content of 8-OHdG in mtDNA, an index for cellular oxidative damage, was determined by QPCR and presented as ΔCt. The degree of oxidative mtDNA damage, $mtDNA^{\Delta Ct}$, was determined as $\Delta Ct = Ct_2$ (hOGG1 treatment) – Ct_1 (no hOGG1 treatment), thus, the larger the ΔCt, the more abundant the 8-OHdG and more oxidative damage present in the sample. Tables 2.4 -2.8 show the oxidative mtDNA damage for each group. The degree of oxidative mtDNA damage (p=0.59) did not significantly differ between male and female neoplastic epilepsy patients (Table 5). Controls had lower oxidative mtDNA damage (0.59) compared to neoplastic epilepsy patients (0.63, p=0.72), MCD (0.63, p=0.84), and non-MCD (0.63, p=0.71) groups, however, statistical significance was not reached. Yet, the degree oxidative mtDNA damage in MCD and non-MCD groups did not differ (both means=0.63, p=0.99).

Interestingly, female neoplastic epilepsy patients presented a slightly higher degree of mtDNA oxidative damage compared to controls (p=0.99) (Table 2.5).

mtDNA genotyping

In this study we analyzed the association between ten mitochondrial SNPs and neoplastic intractable epilepsy (MCD and non-MCD). Neither, neoplastic epilepsy patients (MCD and non-MCD) or controls contained the mtSNP of G9804A. Allele G9952A (p<0.001; Adjusted OR=1.19E9, 95% CI [0, NaN]) was found at higher frequencies in epilepsy samples compared to controls. Controls had higher frequencies for the mitochondrial SNPs A1555G, G3196A, T3197C, A4317G, A10006G, T10010C, and A10398G, and the haplogroups I, J, L, M, and U5 compared to all neoplastic epilepsy samples (Tables 2. 9 and 2.10), including MCD (Tables 2.11 and 2.12) and non-MCD (Tables 2.13 and 2.14) sub-groups.

The neoplastic epilepsy (MCD and non-MCD) samples did not contain any T3197C mtSNP, or belong to the European haplogroup (U-5). The European (I and J), African (L and M) and Asian (M) haplogroups were observed in both neoplastic epilepsy and control samples. No difference in the frequencies of SNPs and haplogroups were observed when comparing MCD and Non-MCD neoplastic epilepsy patient sub-groups (Tables 2.16 and 2.17). Neoplastic males and females presented similar trends in the frequency of SNPs, however, males presented with allele G9804A (Table 2.11), while, females did not (Table 2.13). Interestingly, female neoplastic samples presented higher frequencies of G9952A compared to controls (p=0.02) (Table 2.13).

Logistic regression revealed that neoplastic epilepsy, MCD or non-MCD phenotypes did not significantly depend on level of relative mtDNA copy number, level

of oxidative mtDNA damage, SNP or haplogroup status (Tables 2.16-2.19). A trend that the levels of relative mtDNA copy number and oxidative mtDNA damage and for the SNPs G9952A (OR= 2.94E9, 95% CI [0, NaN]) and T10010C (OR= 1.76, 95% CI [0.28, 11.28]) predicted neoplastic epilepsy phenotype was observed, yet, there were large confidence intervals due large standard error (Table 2.21). Female neoplastic epilepsy phenotypes were predicted by G9952A (OR= 9.91E9, 95% CI [0, NaN]), level of relative mtDNA copy number (4.10, 95% CI [0.28, 60.18]), and level of oxidative mtDNA damage (OR= 2.34, 95% CI [0.20, 27.21]) (Table 2.23).

Bayesian network

In the female samples we used Banjo© software to learn a Bayesian network to study the relationships among epilepsy status, MCD status, relative mtDNA copy number, oxidative mtDNA damage, and the mtSNPs. A summary Bayesian network based on the top three Bayesian networks for the female samples was created (Figure 2.2). In this network brain tumor status is connected to relative mtDNA copy number to oxidative mtDNA damage, and 3 mtDNA SNPs (A10006G, T10010C, and A1555G), as well as, brain tumor with 6 mtDNA SNPs (G9952A, A10398G, A10006G, T3197C, G3196A, and A1555G). The paths of relative mtDNA copy number- oxidative mtDNA copy number- A10006G, G9952A- brain tumor, A10398G-brain tumor, and brain tumor-A1006G, T3197C, G3196A, and A1555G) are shown in the summary Bayesian network. The influence scores between several of these variables are depicted on Table 2.27. The Pearson correlation of between selected variables based on the influence scores are found in Table 2.28. Significant correlations ($p < 0.05$) were found between brain tumor and MCD status; brain tumor with G9952A, T3197C, A10006G, A1555G, and A10398G;

oxidative mtDNA damage with relative mtDNA copy number; brain tumor with relative mtDNA copy number, to name a few. Thus, the summary Bayesian network summarizes the relationship between mtDNA oxidative damage and copy number, and SNPs (genes) and disease (phenotype).

Discussion

One of the major risk factors for cancer is oxidative stress (Bai et al., 2007). Mitochondria play a crucial role in the production of ROS. Mitochondrial dysfunction may contribute to epileptogenesis (Walbaum and Patel, 2010). Oxidative stress has been suggested to be a significant cause and consequence of excitotoxicity, which plays a critical role in epileptic brain damage. mtDNA variations can cause inefficient oxidative phosphorylation which leads to the accumulation of ROS and DNA damage leading to an increased risk in cancer (Bai et al., 2007). In this study we observed a trend of an increased relative mtDNA copy number and oxidative mtDNA damage in neoplastic epilepsy patients (both MCD and non-MCD). Female neoplastic samples presented higher relative mtDNA copy number and degree of oxidative mtDNA damage compared to controls. No significant differences by gender, regarding relative mtDNA copy number and degree of oxidative mtDNA damage, as well as mtDNA SNP and haplogroup background, were observed among neoplastic pediatric epilepsy patients.

The mtDNA variant, G9952A, which codes for COIII and associated with mitochondrial encephalopathy, was found in higher frequencies in the neoplastic intractable epilepsy (MCD and non-MCD) patients, and most interestingly in female neoplastic epilepsy patients. We also found a trend in which G9952A, along with level of mtDNA copy number and level of oxidative mtDNA damage to predicted neoplastic

MCD and non-MCD epilepsy phenotypes, as well as, female neoplastic epilepsy samples. In female samples, a summary Bayesian network revealed brain tumor status is connected to relative mtDNA copy number to oxidative mtDNA damage, and 3 mtDNA SNPs (A10006G, T10010C, and A1555G), as well as, brain tumor with 6 mtDNA SNPs (G9952A, A10398G, A10006G, T3197C, G3196A, and A1555G). Additionally, all of the neoplastic MCDs were gangliomas and DNETs which are malformations due to abnormal glial and neuronal proliferation. A key event in brain development is proliferation which begins around the 20th week of gestation (Lenroot and Giedd, 2006).

Oxidative stress can lead to mutations or the formation of damaged proteins and may be an important risk factor for the initiation and progression of disease (Migliore and Coppede, 2002). Involvement of the mitochondrion in cancer metabolism and functions has been documented (Mayevsky, 2009; Reuter et al., 2010). Increased ROS production contributes to tumorigenicity and cell progression by promoting genomic instability and increased DNA damage. Disturbed redox status may lead to the activation of key signaling components that are important in cell proliferation and survival (Myatt et al., 2011). Changes in glucose metabolism in cancer cells demonstrate a shift away from mitochondrial respiration towards glycolysis for ATP production (Bhrahimi-Horn et al., 2011).

Amplification of the mitochondrial genome in response to oxidative stress has been noted. In a cell, the abundance of mitochondria is determined by biogenesis and division of the organelles. The quantity of mitochondria per cell is tightly regulated by activation of specific transcription factors and signaling pathways (Lee and Wei, 2005). The assembly and functioning of the respiratory enzyme complexes in cells require

coordinated expression the interaction of gene products between the mitochondrial and nuclear genomes. Gene expressions of the mitochondria and nucleus respond in a complex manner to various physiological and developmental signals such as growth activation, neoplastic transformation, muscle contraction, cell differentiation and hormone action (Lee and Wei, 2005). Control of biogenesis in mitochondria is a complex process. Alterations in intracellular level of ROS are associated with changes in mitochondrial abundance, mtDNA copy number, and the expression of respiratory genes. In aging, increased oxidative stress plays a crucial role in the increase of mitochondrial abundance, as well as, mtDNA content in tissue cells during the aging process. Persistent oxidative stress in mitochondria not only contributes to the somatic mtDNA mutations but also alter mtDNA replication rate, leading to an overall decline in the mitochondrial respiratory function.

Replication of mtDNA occurs primarily during the S and G_2 phases of the cell cycle, but most importantly can occur at any point of the cell cycle. In addition, mtDNA replication does not occur concurrently with the growth and division of organelles, hence, mtDNA replication can occur without mitochondrial proliferation. The copy number of mtDNA varies with cell type and is maintained within a range. mtDNA copy number may be modulated according to the energy needs of the cell. Changes in mtDNA copy number in response to exercise and hormone treatment have been observed (Lee and Wei, 2005). Currently, it is unclear how copy number of mtDNA and the abundance of mitochondria are regulated under different physiological and developmental conditions.

According to Liang and Hays (1996), primary oncocytic tumors have shown moderate amounts of increased copy number in mitochondria sequences, treatment of

adenocarcenoma cell line with trihelose or rats with diethylnitrosamine revealed increases in copy number of mtDNA sequences, and primary and cultured gliomas showed substantial alternations in the copy number of mtDNA genome. In Liang and Hays' (1996) study, as well as our study, the mitochondrial genome shows high degrees of amplification, suggesting these increases may be an early event in tumor genesis. The reasons for increases in mtDNA copy number in neoplastic brain cells are unclear. Liang and Hays (1996) suggest this may be due to the general mechanisms of genomic instability throughout the entire tumor cell or because a miscommunication between the nuclear and mitochondrial genomes.

Jiang et al. (2005) found an increase mtDNA content in the saliva of patients with head and neck squamous cell carcinoma (HNSC). An increase in mtDNA copy number has also been observed in chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma (NHL), thyroid cancer, renal oncocytome, colorectal cancer, endometrial cancer, breast cancer (Mambo et al., 2005; Wang et al., 2005; Lan et al., 2008; Radpour et al., 2009; Hosgood III et al., 2010; Shen et al., 2010; Chen et al., 2011). In contrast, decrease in mtDNA content has been reported to be associated with increased risk of renal cancer. Additionally, a decrease in mtDNA copy number in cancer tissue has been found in lung cancer, gastric cancer, hepatocellular carcinoma, type 2 diabetes, cardiomyopathy, and breast cancer (Yin et al., 2004; Lin et al., 2008; Montier et al., 2009). Thus, it seems that changes in mtDNA content is cancer-type specific (Chen et al., 2011).

Oxidative stress stimulates mitochondrial proliferation to meet the energy needs for cell survival including repair of damage and synthesis of new proteins. Yet, oxidative

stress causes excess ROS production resulting in further oxidative damage. The increase of mtDNA copy number is dependent of the level of oxidative stress, the capacity of intracellular antioxidant system, the quality of mitochondria and mtDNA. The increase of ROS production from a defective respiratory chain is thought to play a role in the increase of mitochondrial content. ROS act as a second messenger to trigger the expression of nuclear respiratory factors and mitochondrial transcription factor to induce mitochondrial biogenesis and mitochondrial proliferation (Jiang et al., 2005). Understanding oxidative-stress induced alterations in mtDNA copy number is important for the development of novel drugs to prevent and treat intractable epilepsy.

mtDNA copy numbers can be modulated when physiological conditions are changed. Environmental exposures can generate ROS and may induce the accumulation of mtDNA mutations in human tissue. mtDNA is more susceptible to oxidative damage due to its lack of histones and limited capacity to repair DNA damage, thus, consequently acquires mutations at greater rates than nuclear DNA. Mitochondrial function is compromised as a result of oxidative damage and damage to mtDNA. Oxidative stress stimulates mitochondrial proliferation to meet the energy needs for cell survival including repair of damage and synthesis of new proteins. Oxidative stress causes excess ROS production resulting in further oxidative damage. The increase of mtDNA copy number is dependent of the level of oxidative stress, the capacity of intracellular antioxidant system, the quality of mitochondria and mtDNA. The increase of ROS production from a defective respiratory chain is thought to play a role in the increase of mitochondrial content. ROS act as a second messenger to trigger the expression of nuclear respiratory factors and mitochondrial transcription factor to induce mitochondrial biogenesis and

mitochondrial proliferation (Jiang et al., 2005). Oxidative damage induces an increase in mitochondria and mtDNA to compensate for the decline in the function of mitochondrial respiration (Shen et al., 2008). In other words, ROS resulting from oxidative stress interact with genetic signaling systems that upregulate gene expression to counteract stressor challenges and to re-establish hemeostatis.

Overall, we interpret the increase in relative mtDNA copy number as a result of compensatory responses induced by mtDNA damage, in order to repress the actions of the oxidative damage. The increase in mtDNA replication, as a response to oxidative damage, provides a propagation of mtDNA that has not been damaged by oxidative stress. The resulting mtDNA copies do not contain the damage (DNA adducts), presenting an overall lower oxidative mtDNA damage. Thus, the increase in undamaged mtDNA dilutes the presence of damage mtDNA. Understanding oxidative-stress induced alterations in mtDNA copy number is important for the development of novel drugs to prevent and treat intractable neoplastic epilepsy.

Our study found that the mitochondrial SNP G9952A was found in the female neoplastic epilepsy patients and not the control samples. This mitochondrial variant codes for COIII. It is believed that the majority of the ROS are generated by complexes I and III, likely due to the release of electrons by NADH and FADH into the ETC. Hanna et al. (1998) identified the G9952A point mutation in a patient with encephalopathy and exercise intolerance. This point mutation is located in 3' end of the gene for the subunit of COIII and is thought to result in the loss of the last 13 amino acids of the C-terminal region of this subunit. Decreased expression of cytochrome c oxidase III has been reported in colon tumors compared to non-malignant colonoica mucosa (Penta et al.,

2001). Our results suggest that the presence of these mitochondrial mutations in COIII, in conjunction with environmental insults, may induce mitochondrial dysfunction in neoplastic epilepsy. Hence, the role and mechanisms dysfunction of COIII of the respiratory chain in epileptogenesis and neoplastic MCD needs to be explored further.

In summary, our study shows a trend of increased relative mtDNA copy number in brain tissues from pediatric neoplastic epilepsy patients. The quantitative changes of mtDNA may have implications in neoplastic epilepsy development. Since all of the neoplastic MCDs were gangliomas and DNETs, which are malformations due to abnormal glial and neuronal proliferation, our results suggest key events, such as environmental insults, occurring during this gestational stage and the occurrence of intractable epilepsy warrants further research. In addition, the use of a summary Bayesian network of the female samples demonstrated the statistically significant relationships, the network revealed brain tumor status is connected to relative mtDNA copy number to oxidative mtDNA damage, and 3 mtDNA SNPs (A10006G, T10010C, and A1555G), as well as, brain tumor with 6 mtDNA SNPs (G9952A, A10398G, A10006G, T3197C, G3196A, and A1555G). The summary Bayesian network for the female epilepsy samples revealed the following paths: oxidative mtDNA damage-epilepsy-relative mtDNA copy number, A10398G, T3197C, G3196A, and A1555G; oxidative mtDNA damage-A10006G-epilepsy; oxidative mtDNA damage-relative mtDNA copy number; and G9952A-epilepsy.

To our knowledge, this is the first study to explore mtDNA copy number, oxidative mtDNA oxidative stress in MCD neoplastic epilepsy patients. The main limitation of our study is that the number of pediatric neoplastic intractable epilepsy

patients and non-epilepsy samples is relatively small. However, results from this study provide the foundation, for proper determination of sample size in pediatric intractable epileptics, such as the frequency of mtSNPs in pediatric neoplastic epilepsy patients, and the mean and standard deviation for further research regarding measurement of oxidative mtDNA damage. For example, utilizing the results from this study, in order to compare oxidative mtDNA damage between brain tumor and control samples, and for the study to reach 80% power with an alpha level of 0.05 a sample size of 1766 (883 disease and 883 control samples) is required. Hence, in order to acquire these sample sizes, a multi-facility (and institution) study is needed to explore the oxidative mtDNA damage, mtDNA copy number, and mtSNPs in children with neoplastic intractable epilepsy. In addition to its role in apoptosis, the mitochondrion serves as an important element in the tumorigenic phenotype, and clinical approaches targeting this organelle have potential for the development of effective treatment regimens for patients with neoplastic epilepsy. Overall, this study indicates mitochondria may play a role in neoplastic pediatric epilepsy.

References

Alexander, G.M., and Godwin, D.W. (2006). Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy Research, 71,* 1-22.

Anderson, J.K. (2004). Oxidative stress in neurodegeneration: cause or consequence. *Nature Reviews Neuroscience, 5,* S18-S25.

Bai, R., Leal, S.M., Covarrubias, D. et al. (2007). Mitochondrial genetic background modifies breast cancer risk. *Cancer Research, 67,* 4687-4694.

Bai, R., Wong, L., and Leal, S.M. (2008). Mitochondrial DNA variant interactions modify breast cancer risk. *Journal of Human Genetics, 53,* 924-928.

Barkovich, A.J., Kuzniecky, R.I., Jackson, G.D., et al. (2005). A developmental and genetic classification for malformations of cortical development. *Neurology, 65,* 1873-1887.

Becker, A.J., Urbach, H., Scheffler, B., Baden, T., Normann, S., et al. (2002). Focal cortical dysplasia of Taylor's balloon cell type: Mutational analysis of the TSC1 gene indicates a pathogenic relationship to tuberous sclerosis. *Annals of Neurology, 52,* 29-37.

Brahimi-Horn, M., Bellot, G., and Pouysegur, J. (2011). Hypoxi and energetic tumor metabolism. *Current Opinion in Genetics and Development, 21,* 67-72.

Chang, E.F., Christie, C., Sullivan, J.E., et al. (2010). Seizure control outcomes after resection of dysFpembryoplastic neuroepithelial tumor in 50 patients. *Journal of Neurosurgy: Pediatrics, 5,* 123-130.

Chen, T., He, J., Shen, L., et al. (2011). The mitochondrial DNA 4,977-bp deletion and its implication in copy number alteration in colorectal cancer. *BMC. Medical Genetics, 12,* 8-16

Chuang, Y. (2010). Mitochondrial dysfunction and oxidative stress in seizure-induced neuronal death. *Acta Neurologica Taiwanica, 19,* 3-15.

Devi, P.U., Monocha, A., and Vohora, D. (2008). Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers. *Expert Opinion on Pharmacotherapy, 9,* 3169-3177.

Filiano, J.J., Goldenthal, M.J., Rhodes, H., et al. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. *Journal of Child Neurology, 17,* 435-439.

Govori, V., Humolli, I., Cepreganov, M., et al. (2010). Brain tumors and epilepsy. *Acta Clinica Croatica, 49,* 133-138.

Hader, W.J., Mackay, M., Otsubo, H., Chitoku, S., Weiss, S., et al. (2004). Cortical dysplastic lesions in children with intractable epilepsy: role of complete resection. *Journal of Neurosurgery: Pediatrics, 100,* 110-117.

Hanna, M., Nelson, P., Rahman, S., et al. (1996). Cytochrome c oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. *American Journal of Genetics, 63,* 29-36.

Hosgood III, H., Liu, C., Rothman, D. et al. (2010). Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. *Carcinogenesis, 31,* 847-849.

Hua, Y., and Crino, P.B. (2003). Single cell lineage analysis in human focal cortical dysplasia. *Cerebral Cortex, 13,* 693-699.

Jiang, W., Masayesva, B., Zahurak, M., et al. (2005). Increased mitochondrial DNA content in saliva associated with head and neck cancer. *Human Cancer Biology, 11,* 2486-2491.

Kim, M. M., Clinger, B.G., Ha, P.K., et al. (2004). Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clinical Cancer Research, 10,* 8512-8515.

Lan, Q., Lim, U., Liu, C., et al. (2008). A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Neoplasia, 112,* 4247-4249.

Lee, H., Yin, P., Lin, J., et al. (2005). Mitochondrial genome instability and mtDNA depletion in human cancers. *Annals of the New York Academy of Sciences, 1042,* 109-122.

Lee, H., and Wei, Y. (2005). Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *The International Journal of Biochemistry and Cell Biology, 37,* 822-834.

Liang, B.C., and Hays, L., (1996). Mitochondrial DNA copy number changes in human gliomas. *Cancer Letters, 106,* 167-173.

Mambo, E., Chatterjee, A., Xing, M., et al. (2005). Tumor-specific changes in mtDNA content in human cancer. *International Journal of Cancer, 116,* 921-924.

Mayevsky, A. (2009). Mitochondrial function and energy metabolism in cancer cells: Past overview and future perspectives. *Mitochondrion, 9,* 165-179.

Migliore, L., and Coppede, F. (2002). Genetic and environmental factors in cancer and neurodegenerative diseases. *Mutation Research, 512,* 135-153.

Montier, L., Deng, J., and Bai, Y. (2009). Number matters: control of mammalian mitochondrial DNA copy number. *Journal of Genetics and Genomics, 36,* 125-131.

Myatt, S., Brosens, J., and Lam, E. (2011). Sense and sensibility: FOXO and ROS in cancer development and treatment. *Antioxidants and Redox Signaling, 14,* 675-687.

Penta, J., Johnson, F., Wachsman, J., et al. (2001). Mitochondrial DNA in human malignancy. *Mutation Research, 488,* 119-133.

Radpour, R., Fan, A., Kohler, C., et al. (2009). Current understanding of mitochondrial DNA in breast cancer. *The Breast Journal, 15,* 505-509.

Reuter, S., Subash, C., Gupta, C., et al. (2010). Oxidative stress, inflammation, and cancer. How are they linked? *Free Radical Biology and Medicine, 49,* 1603-1616.

Rickert, C.H. (2006). Cortical dysplasia: neuropathological aspects. *Child's Nervous System. 22,* 821-826.

Shen, M., Zhang, L., Bonner, M., et al. (2008). Association between mitochondrial DNA copy number, blood cell counts and occupational benzene exposure. *Environmental and Molecular Mutagenesi, 49,* 453-457.

Shen, J., Platek, M., Mahasneh, A., et al. (2010). Mitochondrial copy number and risk of breast cancer. *Mitochondrion. 10*, 62-68.

Schick, V., Majores, M., Koch, A., Elger, C.E. , Schramm, J. et al. (2007a). Alterations of phosphatidylinositol 3-kinase pathway components in epilepsy-associated glioneuronal lesions. *Epilepsia,48,* 65-73.

Schwartzkroin, P.A., and Walsh, C.A. (2000). Cortical malformations and epilepsy. *Mental Retardation and Developmental Diagnosis, 6,* 268-280.

Shokolenko, I., Venediktova, N., Bockkareva, G.L., et al. (2009). Oxidative stress induces degeneration of mitochondrial DNA. *Nucleic Acids Research, 37,* 2539-2548.

Spalice, A., Ruggieri, M., Grosso, S., et al. (2010). Dysembryoplastic neuronal tumors: A prospective clinicopathologic and outcome study of 13 children. *Pediatric Neurology, 43,* 395-402.

Sugimoto, T., Mori, C., Takanami, T., Sasagawa, Y., Saito, R., et al. (2008). Caenorhabditis elegans par21/mtssb-1 is essential for mitochondrial DNA replication and its defects causes comprehensive transcriptional alterations including a hypoxia response. *Experimental Cell Research, 314,* 103-114.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J.M., Rhee, J. et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127,* 397-408.

Szuhai, K.S., van den Ouweland, J.M., Dirks, R.W. et al. (2001). Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quantification in Myoclonus Epilepsy and Ragged-Red Fibers (MERRF) syndrome by multiplex Molecular Beacon based real-time fluorescence PCR. *Nucleic Acids Research, 29,* e13.

Tanaka, N., Goto, Y., Akanuma, J. et al. (2010). Mitochondrial DNA variants in a Japanese population of patients with Alzheimer's disease. *Mitochondrion, 10,* 32-37.

Tsutsui, H., Ide, T., Shiomi, T., et al. (2001). 8-Oxo-dGTPase, which prevents oxidative stress-induced DNA damage, increases in the mitochondrion from failing hearts. *Circulation, 104,* 2883-2885.

Tzen, C., Mau, B., and Hsu, H. (2007). Analysis of disease-associated ND4 mutations: How do we know which mutation is pathogenic. *Mitochondrion, 7,* 151-156.

Waldbaum, S., and Patel, M. (2010a). Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy. *Journal of Bioenergetics and Biomembranes, 42,* 449-455.

Waldbaum, S., and Patel, M. (2010b). Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Research, 88,* 22-45.

Wang, Y., Liu, V., Xue, W., et al. (2005). The increase of mitochondrial DNA content in endometrial adenocarcinoma cells: A quantitative study using laser-captured microdissected tissues. *Gyencologic Oncology, 98,* 104-110.

Wiseman, H., and Halliwell, B. (1995). Damage to DNA by reactive oxygen species and nitrogen species role in inflammatory disease and progression to cancer. *Biochemical Journal, 313,* 17-29.

Wong, M. (2007). Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia, 48,* 617-630.

Wosik, K., Ruffini, F., Almazan, G., Olivera, A., Nalbantoglu, J.L. et al. (2004). Resistance of human adult oligodendrocytes to AMPA/kainite receptor-mediated glutamate injury. *Brain, 127,* 2636-2648.

Yasin, S.A., Latak, K., Becherini, F., et al. (2010). Balloon cells in human cortical dysplasia and tuberous sclerosis: isolation of a pathological progenitor-like cell. *Acta Neuropathologica, 120,* 85-96.

Yin, P., Lee, H., Chau, G., et al. (2004). Alterations of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. *British Journal of Cancer, 90*, 2390-2396.

Table 2.1.
Clinical profile of pediatric patients with intractable epilepsy with brain tumors

Sample	Age (years)	Gender	Diagnosis	MCD Status	Pathology
1	3	M	tumor	Non-MCD	new tumor dense gliosis
2	9	F	tumor	Non-MCD	pilocytic astrocytoma
3	14	M	tumor	MCD	ganglioglioma
4	8 ms	F	tumor	MCD	ganglioglioma
5	8	M	tumor	Non-MCD	oligodendroglioma
6	12	F	tumor	Non-MCD	pilocytic astrocytoma
8	11	F	tumor	Non-MCD	pilocytic astrocytoma
12	18	M	tumor	MCD	Dysembryoplastic Neuroepithelial Tumor (DNET)
13	12	F	tumor	Non-MCD	glioblastoma multiforme
14	10	F	tumor	Non-MCD	medulloblastoma
15	10	M	tumor	Non-MCD	pilocytic astrocytoma
16	8 mos	M	tumor	Non-MCD	astrocytoma
17	6	F	tumor	Non-MCD	choroid plexus papilloma
19	5	M	tumor	Non-MCD	pilocytic astrocytoma
21	10	F	tumor	MCD	ganglioglioma
22	6	M	tumor	Non-MCD	pilocytic astrocytoma, glioblastoma, hypothalamic tumor
26	19	F	tumor	Non-MCD	oligodendroglioma
28	2	M	tumor	Non-MCD	medulloblastoma
29	4	F	tumor	Non-MCD	pilocytic astrocytoma, hypothalamic tumor
30	5	M	tumor	MCD	glioneuronal neoplasm/possible ganglioglioma
32	9	F	tumor	Non-MCD	pilocytic astrocytoma
35	13	M	tumor	Non-MCD	medulloblastoma
36	16	F	tumor	Non-MCD	ependymoma
39	12	F	tumor	Non-MCD	medulloblastoma
41	7	M	tumor	Non-MCD	anaplastic ependymoma
44	6	F	tumor	Non-MCD	high grade malignant undifferentiated neoplasm
46	4	F	tumor	MCD	DNET

Table 2.2
Profile of control samples obtained from NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland

Sample	Age (years)	Gender	History/ Cause of Death
Control 1	15	F	car accident, multiple injuries
Control 2	18	F	car accident, multiple injuries
Control 3	13	F	asphyxia by hanging
Control 4	2	F	drowning
Control 5	2	F	drowning
Control 6	8	F	asphyxia and multiple injuries
Control 7	4	F	lymphocytic myocarditis
Control 8	16	F	car accident, multiple injuries
Control 9	2	F	car accident, multiple injuries
Control 10	17	F	car accident, multiple injuries
Control 11	10	F	Asthma

Table 2.3.
Mitochondrial SNPs and haplogroups used in this study

Variant	Region	Reported in Patients
A1555G	12S rRNA	Maternally inherited deafness or aminoglycoside-induced deafness
G3196A	16S rRNA	Alzheimer's Disease and Parkinson's Disease (ADPD)
T3197C	16S rRNA	Haplogroup (Hg)-U5
G4309A	tRNA Ile	Chronic Progressive External Ophthalmoplegia (CPEO)
A4317G	tRNA Ile	Fatal Infantile Cardiomyopathy (FICP)
G9804A	CO III	Leber Hereditary Optic Neuropathy (LHON)
G9952A	CO III	Mitochondrial Encephalopathy
A10006G	tRNA Gly	Chronic intestinal pseudo-obstruction (CIPO)
T10010C	tRNA Gly	Progressive encephalopathy (PEM)
A10398G	ND3	↓PD, ↓AD;A-↑Breast Cancer (BRCA) in AA, Hg-I, J, L, M

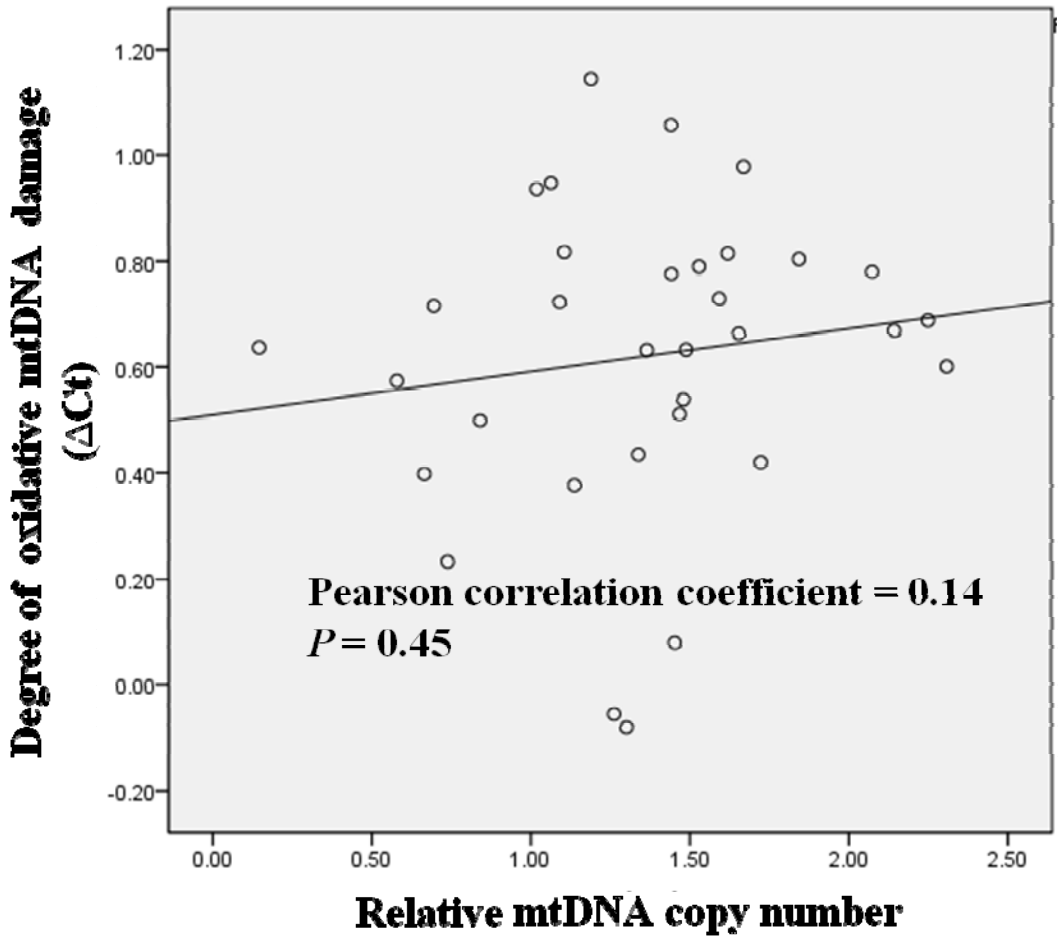

Figure 2.1.

Correlation between the mtDNA copy number and degree of oxidative mtDNA damage. The relative mtDNA copy numbers of the intractable epilepsy neoplastic tissues and control tissues with their relationship to degree of oxidative mtDNA damage are plotted. The results show that the two parameters were not significantly correlated (Pearson correlation coefficient= 0.14, p= 0.45).

Table 2.4.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=38)	Neoplastic epilepsy (n=27)	Control (n=11)	*P*-value
Age (years)	8.93 ± 5.36	6.96 ± 5.37	9.73 ± 6.44	0.24
Sex (M/F)	12/26	12/15	0/11	<0.001
log Relative mtDNA copy number				
Median	1.45	1.45	1.37	
Mean ± S.D.	1.53 ± 1.00	1.61 ± 1.18	1.33 ± 0.21	0.26
> 1.45 (high) (%)		14 (52)	5 (45)	1.00
≤ 1.45 (low) (%)		13 (48)	6 (55)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.66	0.68	0.63	
Mean ± S.D.	0.62 ± 0.29	0.63 ± 0.30	0.59 ± 0.27	0.72
> 0.66 (high damage) (%)		12 (44)	5 (45)	0.72
≤ 0.66 (low damage) (%)		10 (37)	6 (55)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.46	0.48	0.42	
Mean ± S.D.	0.60 ± 0.73	0.67 ± 0.87	0.46 ± 0.25	0.44
> 0.46 (high damage) (%)		11 (41)	6 (55)	1.00
≤ 0.46 (low damage) (%)		11 (41)	4 (36)	

Table 2.5.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank by gender

	All Cases (n=38)	Epilepsy Male (n=12)	Epilepsy Female (n=15)	Control Female (n=11)	[a]*P*-value	[b]*P*-value	[c]*P*-value
Age (years)	8.93 ± 5.36	7.64 ± 5.23	9.38 ± 4.76	9.73 ± 6.44	0.38	0.40	0.87
log Relative mtDNA copy number	12/26						
Median		1.44	1.48	1.37			
Mean ± S.D.	1.45	1.47 ± 0.68	1.55 ± 1.14	1.33 ± 0.21	0.82	0.52	0.34
> 1.45 (high) (%)	1.53 ± 1.00	4 (33)	9 (60)	0 (0)	0.48	1.00	0.69
≤ 1.45 (low) (%)		7 (58)	6 (40)	11 (100)			
log Degree of oxidative mtDNA damage (ΔCt)							
Median	0.66	0.69	0.65	0.63			
Mean ± S.D.	0.62 ± 0.29	0.66 ± 0.31	0.60 ± 0.28	0.59 ± 0.27	0.59	0.61	0.72
> 0.66 (high damage) (%)		4 (33)	6 (40)	5 (45)	0.67	1.00	0.67
≤ 0.66 (low damage) (%)		6 (50)	5 (33)	6 (55)			
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number							
Median	0.46	0.59	0.44	0.42			
Mean ± S.D.	0.60 ± 0.73	0.57 ± 0.31	0.78 ± 1.21	0.46 ± 0.25	0.84	0.41	0.41
> 0.46 (high damage) (%)		7 (58)	4 (27)	4 (36)	1.00	0.40	1.00
≤ 0.46 (low damage) (%)		4 (33)	7 (47)	6 (55)			

[a]P-value for Neoplastic Epilepsy, Male vs. Neoplastic Epilepsy, Female
[b]P-value for Neoplastic Epilepsy, Neoplastic Male vs. Control
[c]P-value for Neoplastic Epilepsy, Female vs. Control

Table 2.6.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=17)	MCD (n=6)	Control (n=11)	*P*-value
Age (years)	9.33 ± 6.3	6.96 ± 5.37	9.73 ± 6.44	0.24
Sex (M/F)	3/14	3/3	0/11	0.03
log Relative mtDNA copy number				
Median	1.44	1.85	1.37	
Mean ± S.D.	1.58 ± 0.66	2.03 ± 0.97	1.33 ± 0.21	0.14
> 1.44 (high) (%)		4 (67)	5 (45)	0.62
≤ 1.44 (low) (%)		2 (33)	6 (55)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.65	0.69	0.63	
Mean ± S.D.	0.61 ± 0.32	0.63 ± 0.44	0.59 ± 0.27	0.84
> 0.65 (high damage) (%)		3 (50)	5 (45)	1.00
≤ 0.65 (low damage) (%)		2 (33)	6 (55)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.41	0.3	0.42	
Mean ± S.D.	0.45 ± 0.29	0.40 ± 0.37	0.46 ± 0.25	0.71
> 0.40 (high damage) (%)		3 (50)	6 (55)	1.00
≤ 0.40 (low damage) (%)		2 (33)	4 (36)	

Table 2.7.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=32)	Non-MCD (n=21)	Control (n=11)	*P*-value
Age (years)	8.99 ± 5.22	8.60 ± 4.59	9.73 ± 6.44	0.51
Sex (M/F)	9/23	9/12	0/11	0.01
log Relative mtDNA copy number				
Median	1.45	1.45	1.37	
Mean ± S.D.	1.43 ± 1.00	1.48 ± 1.23	1.33 ± 0.21	0.59
> 1.45 (high) (%)		11 (52)	0 (0)	1.00
≤ 1.45 (low) (%)		10 (48)	11 (100)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.65	0.66	0.63	
Mean ± S.D.	0.61 ± 0.26	0.63 ± 0.26	0.59 ± 0.27	0.71
> 0.65 (high damage) (%)		9 (43)	5 (45)	1.00
≤ 0.65 (low damage) (%)		8 (38)	6 (55)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.45	0.5	0.42	
Mean ± S.D.	0.64 ± 0.77	0.75 ± 0.96	0.46 ± 0.25	0.34
> 0.45 (high damage) (%)		9 (43)	6 (55)	0.70
≤ 0.45 (low damage) (%)		7 (33)	4 (36)	

Table 2.8.
Comparison of demographic, mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-MCD

	All Cases (n=27)	MCD (n=6)	Non-MCD (n=21)	*P*-value
Age (years)	8.60 ± 4.96	8.61 ± 6.58	8.60 ± 4.59	1.00
Sex (M/F)	12/15	12/9	3/3	1.00
log Relative mtDNA copy number				
Median	1.45	1.85	1.45	
Mean ± S.D.	1.61 ± 1.18	2.03 ± 0.97	1.48 ± 1.23	0.33
> 1.45 (high) (%)		3 (50)	10 (48)	1.00
≤ 1.45 (low) (%)		3 (50)	10 (48)	
log Degree of oxidative mtDNA damage (ΔCt)				
Median	0.68	0.69	0.67	
Mean ± S.D.	0.63 ± 0.30	0.63 ± 0.44	0.63 ± 0.27	0.99
> 0.68 (high damage) (%)		2 (33)	8 (38)	1.00
≤ 0.68 (low damage) (%)		2 (33)	9 (43)	
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number				
Median	0.48	0.31	0.50	
Mean ± S.D.	0.67 ± 0.87	0.40 ± 0.37	0.75 ± 0.96	0.45
> 0.48 (high damage) (%)		2 (33)	9 (43)	1.00
≤ 0.48(low damage) (%)		3 (50)	8 (38)	

Table 2.9.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable neoplastic epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Neoplastic epilepsy (n=27)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	13	48	11	100	0.002	0	0, NaN	0	0, NaN
G3196A	3	11	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	26	96	11	100	1.00	0	0, NaN	0	0, NaN
A4317G	19	70	11	100	0.08	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	NaN	0, NaN	NaN	0, NaN
G9952A	6	22	0	0	<0.001	1.19E9	0, NaN	1.17E9	0, NaN
A10006G	6	22	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	16	59	7	64	1.00	0.83	0.20, 3.54	0.83	0.19, 3.55
A10398G	7	26	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.10.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable neoplastic epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Neoplastic epilepsy (n=27)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	7	26	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	7	26	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	7	26	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	7	26	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.11.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy male neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Neoplastic Epilepsy Male (n=12)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	8	67	11	100	0.09	0	0, NaN	0	0, NaN
G3196A	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	<0.001	0	0, NaN	0	0, NaN
G4309A	11	92	11	100	1.0	0	0, NaN	0	0, NaN
A4317G	9	75	11	100	0.22	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	Infinity	0, NaN	Infinity	0, NaN
G9952A	6	50	0	0	0.01	Infinity	0, NaN	Infinity	0, NaN
A10006G	4	33	11	100	0.001	0	0, NaN	0	0, NaN
T10010C	8	67	7	64	1.00	1.14	0.21, 6.37	1.23	0.21, 7.14
A10398G	3	25	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.12.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy male neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Neoplastic Epilepsy Male (n=12)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	3	25	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	3	25	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	3	25	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	3	25	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.13.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric intractable epilepsy female neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Neoplastic Epilepsy Female (n=15)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	5	33	11	100	0.001	0	0, NaN	0	0, NaN
G3196A	3	20	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	<0.001	0	0, NaN	0	0, NaN
G4309A	15	100	11	100	1.00	NaN	NaN, NaN	NaN	NaN, NaN
A4317G	10	67	11	100	0.05	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	NaN	NaN, NaN	NaN	NaN, NaN
G9952A	6	40	0	0	0.02	1.97E9	0, NaN	2.04E9	0, NaN
A10006G	2	13	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	8	53	7	64	0.70	0.63	0.13, 3.21	0.60	0.13, 3.20
A10398G	4	27	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.14.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric intractable epilepsy female neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Neoplastic Epilepsy Female (n=15)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	4	27	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	4	27	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	4	27	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	4	27	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds Ratio using control group as reference
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.15.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	MCD (n=6)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	2	33	11	100	0.01	0	0, NaN	0	0, NaN
G3196A	1	14	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	6	100	11	100	1.00	NaN	NaN, NaN	NaN	NaN, NaN
A4317G	3	50	11	100	0.03	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	NaN	NaN, NaN	NaN	NaN, NaN
G9952A	3	50	0	0	0.03	5.92E9	0, NaN	1.02E10	0, NaN
A10006G	2	33	11	100	0.01	0	0, NaN	0	0, NaN
T10010C	4	67	7	64	1.00	1.14	0.14, 9.30	1.25	0.15, 10.81
A10398G	2	33	11	100	0.01	0	0, NaN	0	0, NaN

[a]OR, Crude Odds ratio using control group as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.16.
The results of the Fisher's exact test for individual haplogroups for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	MCD (n=6)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	2	33	11	100	0.01	0	0, NaN	0	0, NaN
Hg-J	2	33	11	100	0.01	0	0, NaN	0	0, NaN
Hg-L	2	33	11	100	0.01	0	0, NaN	0	0, NaN
Hg-M	2	33	11	100	0.01	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds ratio using control group as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.17.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	non-MCD (n=21)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	11	52	11	100	0.01	0	0, NaN	0	0, NaN
G3196A	2	10	11	100	<0.001	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	20	95	11	100	1.00	0	0, NaN	0	0, NaN
A4317G	16	76	11	100	0.14	0	0, NaN	0	0, NaN
G9804A	0	0	0	0	1.00	NaN	NaN, NaN	NaN	0, NaN
G9952A	9	43	0	0	0.01	1.48E9	0, NaN	1.44E9	0, NaN
A10006G	4	19	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	12	57	7	64	1.00	0.76	0.17, 3.42	0.72	0.16, 3.31
A10398G	5	24	11	100	<0.001	0	0, NaN	1.05	0.91, 1.21

[a]OR, using control group as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.18.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable neoplastic epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	non-MCD (n=21)		Controls (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	5	24	11	100	<0.001	0	0, NaN	0.95	0.91, 1.21
Hg-J	5	24	11	100	<0.001	0	0, NaN	1.05	0.91, 1.21
Hg-L	5	24	11	100	<0.001	0	0, NaN	1.05	0.91, 1.21
Hg-M	5	24	11	100	<0.001	0	0, NaN	0.95	0.91, 1.21
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, using control group as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.19.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-MCD

SNPs	MCD (n=6)		Non-MCD (n=21)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	2	33	11	52	0.65	0.45	0.07, 3.04	0.42	0.32, 17.58
G3196A	1	17	2	10	0.55	1.90	0.14, 25.45	0.52	0.04, 7.19
T3197C	0	0	0	0	1.00	NaN	NaN, NaN	1.00	0.83, 1.21
G4309A	6	100	20	95	1.00	0	0, NaN	0	0, NaN
A4317G	3	50	16	76	0.32	0.31	0.05, 2.07	0.31	0.48, 22.13
G9804A	0	0	0	0	1.00	NaN	NaN, NaN	NaN	NaN, NaN
G9952A	3	50	9	43	1.00	1.33	0.22, 8.22	0.75	0.12, 4.62
A10006G	2	33	4	19	0.59	2.13	0.28, 15.97	0.46	0.06, 3.57
T10010C	4	67	12	57	1.00	1.50	0.22, 10.08	0.67	0.10, 4.48
A10398G	2	33	5	24	0.63	1.60	0.22,11.50	0.62	0.09, 4.50

[a]OR, Crude Odds ratio using non-MCD as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.20.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients with MCD and non-MCD

Haplogroups	MCD (n=6)		Non-MCD (n=21)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	2	33	5	24	0.63	1.60	0.22,11.50	0.62	0.09, 4.50
Hg-J	2	33	5	24	0.63	1.60	0.22,11.50	0.62	0.09, 4.50
Hg-L	2	33	5	24	0.63	1.60	0.22,11.50	0.62	0.09, 4.50
Hg-M	2	33	5	24	0.63	1.60	0.22,11.50	0.62	0.09, 4.50
Hg-U5	0	0	0	0	1.00	NaN	NaN, NaN	1.00	0.04, 7.19

[a]OR, Crude Odds ratio using non-MCD as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table 2.21.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Haplogroup	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	2.21 (0.34, 20.90)	2.59 (0.37, 18.28)	1.06 (0.89, 1.27)
G3196A	---	---	---	0.92 (0.60, 1.39)
T3197C	---	2.02E8 (0, NaN)	0.89 (0.03, 25.33)	1.06 (0.78, 1.42)
G4309A	---	1.00 (0.19, 5.43)	1.93 (0.40, 9.37)	1.02 (0.88, 1.19)
A4317G	---	1.15 (0.19, 6.80)	1.51 (0.29, 7.87)	1.01 (0.87, 1.18)
G9804A	---	1.00 (0.19, 5.43)	1.93 (0.40, 9.37)	1.02 (0.88, 1.19)
G9952A	2.94E9 (0, NaN)	3.67 (0.43, 31.22)	1.93 (0.29, 13.03)	1.04 (0.87, 1.25)
A10006G	0 (0, NaN)	0 (0, NaN)	0.25 (0.01, 6.04)	0.95 (0.75, 1.21)
T10010C	1.76 (0.28, 11.18)	1.33 (0.19, 9.41)	1.88 (0.39, 9.16)	1.03 (0.88, 1.20)
A10398G	---	0.87 (0.9, 8.67)	3.67 (0.36, 37.07)	1.06 (0.85, 1.31)
Hg-I	---	0.87 (0.9, 8.67)	3.67 (0.36, 37.07)	1.06 (0.85, 1.31)
Hg-J	---	0.87 (0.9, 8.67)	3.67 (0.36, 37.07)	1.06 (0.85, 1.31)
Hg-L	---	0.87 (0.9, 8.67)	3.67 (0.36, 37.07)	1.06 (0.85, 1.31)
Hg-M	---	0.87 (0.9, 8.67)	3.67 (0.36, 37.07)	1.06 (0.85, 1.31)
Hg-U5	---	2.02E8 (0, NaN)	0.89 (0.03, 25.33)	1.06 (0.78, 1.42)

OR, using control group as reference
NaN = Not a number

Table 2.22.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric intractable epilepsy male neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	0.41 (0.03, 6.73)	0.74 (0.07, 8.51)	1.10 (0.89, 1.37)
G3196A	---	---	---	---
T3197C	0 (0, NaN)	1.53E8 (0, NaN)	1.63 (0.05, 53.48)	1.05 (0.78, 1.40)
G4309A	---	0.36 (0.04, 3.10)	0.98 (0.13, 7.33)	1.02 (0.87, 1.20)
A4317G	0 (0, NaN)	0.54 (0.06, 5.18)	1.03 (0.12, 8.91)	1.04 (0.89, 1.23)
G9804A	---	0.36 (0.04, 3.10)	0.98 (0.13, 7.33)	1.02 (0.87, 1.20)
G9952A	9.79E9 (0, NaN)	1.58 (0.10, 24.97)	0.66 (0.04, 11.80)	1.13 (0.89, 1.43)
A10006G	0 (0, NaN)	0.50 (0.03, 8.85)	0.59 (0.03, 12.06)	0.95 (0.76, 1.20)
T10010C	1.44 (0.14, 14.52)	0.42 (0.04, 4.62)	1.04 (0.14, 7.87)	1.03 (0.87, 1.21)
A10398G	0 (0, NaN)	0 (0, NaN)	0.75 (0.02, 23.25)	0.92 (0.68, 1.25)
Hg-I	0 (0, NaN)	0 (0, NaN)	0.75 (0.02, 23.25)	0.92 (0.68, 1.25)
Hg-J	0 (0, NaN)	0 (0, NaN)	0.75 (0.02, 23.25)	0.92 (0.68, 1.25)
Hg-L	0 (0, NaN)	0 (0, NaN)	0.75 (0.02, 23.25)	0.92 (0.68, 1.25)
Hg-M	0 (0, NaN)	0 (0, NaN)	0.75 (0.02, 23.25)	0.92 (0.68, 1.25)
Hg-U5	0 (0, NaN)	1.53E8 (0, NaN)	1.63 (0.05, 53.48)	1.05 (0.78, 1.40)

OR, using control group as reference
NaN = Not a number

Table 2.23.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric intractable epilepsy female neoplastic patients and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Hg	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	1.49E19 (0, NaN)	5.01E23 (0, NaN)	0 (0, NaN)
G3196A	0 (0, NaN)	0 (0, NaN)	0 (0, NaN)	0.92 (0.60, 1.39)
T3197C	0 (0, NaN)	6.91E8 (0, NaN)	0.30 (0.02, 37.41)	1.03 (0.65, 1.61)
G4309A	---	1.33 (0.20, 8.68)	2.07 (0.34, 12.79)	1.01 (0.84, 1.21)
A4317G	0 (0, NaN)	1.23 (0.16, 9.99)	1.04 (0.13, 8.30)	0.94 (0.77, 1.15)
G9804A	---	1.31 (0.20, 8.68)	2.07 (0.34, 12.79)	1.01 (0.84, 1.21)
G9952A	9.91E9 (0, NaN)	4.10 (0.28, 60.18)	2.34 (0.20, 27.21)	0.94 (0.74, 1.20)
A10006G	0 (0, NaN)	0 (0, NaN)	0 (0, NaN)	1.20 (0.73, 1.97)
T10010C	1.71 (0.24, 12.04)	1.53 (0.22, 10.88)	1.97 (031, 12.42)	1.01 (0.84, 1.21)
A10398G	0 (0, NaN)	2.45 (0.15, 40.13)	10.58 (0.41, 271.59)	1.17 (0.85, 1.62)
Hg-I	0 (0, NaN)	2.45 (0.15, 40.13)	10.58 (0.41, 271.59)	1.17 (0.85, 1.62)
Hg-J	0 (0, NaN)	2.45 (0.15, 40.13)	10.58 (0.41, 271.59)	1.17 (0.85, 1.62)
Hg-L	0 (0, NaN)	2.45 (0.15, 40.13)	10.58 (0.41, 271.59)	1.17 (0.85, 1.62)
Hg-M	0 (0, NaN)	2.45 (0.15, 40.13)	10.58 (0.41, 271.59)	1.17 (0.85, 1.62)
Hg-U5	0 (0, NaN)	6.91E8 (0, NaN)	0.30 (0.02, 37.41)	1.03 (0.65, 1.61)

OR, using control group as reference
NaN = Not a number

Table 2.24.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Haplogroup	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	2.12E29 (0, NaN)	4.90E29 (0, NaN)	5.47E4 (0, NaN)
G3196A	---	---	---	---
T3197C	0 (0, NaN)	7.64E9(0, NaN)	2768 (0, 1.13E27)	1.63 (0.06, 46.68)
G4309A	---	1.89 (0.21, 17.01)	1.91 (0.21, 17.45)	1.02 (0.85, 1.23)
A4317G	0 (0, NaN)	3.07 (0.17, 54.19)	3.00 (0.17, 51.86)	0.96 (0.75, 1.22)
G9804A	---	1.89 (0.21, 17.01)	1.91 (0.21, 17.45)	1.02 (0.85, 1.23)
G9952A	2.85E18 (0, NaN)	7.72E8 (0, NaN)	1.19 (0.04, 38.39)	1.16 (0.81, 1.65)
A10006G	0 (0, NaN)	0 (0, NaN)	0.88 (0.03, 31.17)	0.98 (0.76, 1.26)
T10010C	3.76 (0.21, 65.95)	3.00 (0.26, 35.00)	1.72 (0.16, 18.42)	1.05 (0.85, 1.29)
A10398G	0 (0, NaN)	1.73 (0.06, 49.31)	1.50 (0.06, 37.91)	1.09 (0.81, 1.47)
Hg-I	0 (0, NaN)	1.73 (0.06, 49.31)	1.50 (0.06, 37.91)	1.09 (0.81, 1.47)
Hg-J	0 (0, NaN)	1.73 (0.06, 49.31)	1.50 (0.06, 37.91)	1.09 (0.81, 1.47)
Hg-L	0 (0, NaN)	1.73 (0.06, 49.31)	1.50 (0.06, 37.91)	1.09 (0.81, 1.47)
Hg-M	0 (0, NaN)	1.73 (0.06, 49.31)	1.50 (0.06, 37.91)	1.09 (0.81, 1.47)
Hg-U5	0 (0, NaN)	7.64E9(0, NaN)	2768 (0, 1.13E27)	1.63 (0.06, 46.68)

OR, using control group as reference
NaN = Not a number

Table 2.25.
The results of the logistic regression for SNP/ haplogroups, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with non-MCD and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Haplogroup	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	0 (0, NaN)	1.58(0.20, 12.28)	1.30 (0.20, 8.38)	1.05 (0.89, 1.24)
G3196A	0 (0, NaN)	0 (0, NaN)	0 (0, NaN)	0.92 (0.60, 1.39)
T3197C	0 (0, NaN)	4.69E8 (0, NaN)	0.55 (0.21, 14.51)	1.13 (0.81, 1.59)
G4309A	---	1.14 (0.22, 6.12)	1.36 (0.28, 6.55)	1.04 (0.89, 1.21)
A4317G	0 (0, NaN)	0.89 (0.14, 5.76)	1.24(0.23, 6.70)	1.02 (0.87, 1.19)
G9804A	---	1.15 (0.22, 6.12)	1.36 (0.28, 6.55)	1.04 (0.89, 1.21)
G9952A	2.86E9 (0, NaN)	1.93 (0.25, 15.03)	1.85 (0.28, 12.28)	1.01 (0.85, 1.20)
A10006G	0 (0, NaN)	1.47 (0.12, 17.63)	0.41 (0.03, 5.92)	1.14 (0.90,1.44)
T10010C	1.34 (0.24, 7.54)	1.25 (0.22, 7.18)	1.31 (0.27, 6.37)	1.03 (0.89, 1.21)
A10398G	0 (0, NaN)	2.06 (0.22, 19.30)	2.10 (0.22, 20.34)	1.07 (0.86, 1.33)
Hg-I	0 (0, NaN)	2.06 (0.22, 19.30)	2.10 (0.22, 20.34)	1.07 (0.86, 1.33)
Hg-J	0 (0, NaN)	2.06 (0.22, 19.30)	2.10 (0.22, 20.34)	1.07 (0.86, 1.33)
Hg-L	0 (0, NaN)	2.06 (0.22, 19.30)	2.10 (0.22, 20.34)	1.07 (0.86, 1.33)
Hg-M	0 (0, NaN)	2.06 (0.22, 19.30)	2.10 (0.22, 20.34)	1.07 (0.86, 1.33)
Hg-U5	0 (0, NaN)	4.69E8 (0, NaN)	0.55 (0.21, 14.51)	1.13 (0.81, 1.59)

OR, using control group as reference
NaN = Not a number

Table 2.26.
The results of the logistic regression for SNP/haplogroups, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with MCD and using non-MCD as control group

SNP/Haplogroup	Haplogroup OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	6.36E8 (0, NaN)	2.43 (0.16, 37.63)	1.08 (0.04, 30.30)	1.10 (0.73, 1.63)
G3196A	9.21E8 (0, NaN)	4.31 (0.28, 67.47)	1.56 (0.11, 21.76)	1.14 (0.86, 1.50)
T3197C	---	3.65 (0.24, 56.02)	1.19 (0.09, 15.19)	1.12 (0.85, 1.47)
G4309A	---	3.65 (0.24, 56.02)	1.19 (0.09, 15.19)	1.12 (0.85, 1.47)
A4317G	4.43 (0.25, 78.98)	3.17 (0.20, 49.63)	2.41 (0.08, 75.88)	1.19 (0.82, 1.71)
G9804A	---	3.65 (0.24, 56.02)	1.19 (0.09, 15.19)	1.12 (0.85, 1.47)
G9952A	0.41 (0.02, 8.47)	2.11 (0.08, 53.18)	1.44 (0.09, 22.05)	1.11 (0.84, 1.48)
A10006G	0.21 (0.01, 3.36)	2.45 (0.15, 40.19)	0.77 (0.04, 15.25)	1.10 (0.84, 1.45)
T10010C	0 (0, NaN)	1.92 (0.13, 28.45)	1.57 (0.08, 31.52)	1.19 (0.83, 1.70)
A10398G	0.23 (0.01, 4.03)	3.17 (0.20, 49.63)	2.41 (0.8, 75.88)	1.19 (0.82, 1.71)
Hg-I	0.23 (0.01, 4.03)	3.17 (0.20, 49.63)	2.41 (0.8, 75.88)	1.19 (0.82, 1.71)
Hg-J	0.23 (0.01, 4.03)	3.17 (0.20, 49.63)	2.41 (0.8, 75.88)	1.19 (0.82, 1.71)
Hg-L	0.23 (0.01, 4.03)	3.17 (0.20, 49.63)	2.41 (0.8, 75.88)	1.19 (0.82, 1.71)
Hg-M	0.23 (0.01, 4.03)	3.17 (0.20, 49.63)	2.41 (0.8, 75.88)	1.19 (0.82, 1.71)
Hg-U5	---	3.65 (0.24, 56.02)	1.19 (0.09, 15.19)	1.12 (0.85, 1.47)

NaN = Not a number

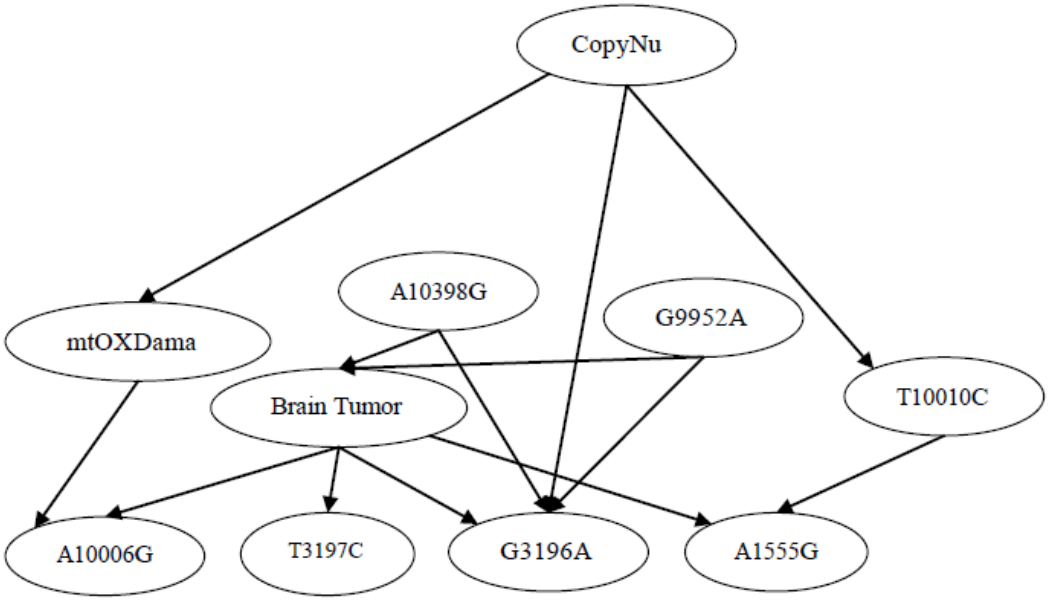

Figure 2.2.

Summary Beyesian network based on the top three Bayesian networks for female samples. Absolute value on influence score higher than 0.1 are shown.

Table 2.27.
Influence scores between selected variables for female samples.

Variables	Influence Score
(BrainTumor) -> (T3197C)	0.7882
(BrainTumor) -> (A10006G)	0.7407
(BrainTumor) -> (A1555G)	0.6156
(A10398G) -> (BrainTumor)	0.4746
(G9952A) -> (BrainTumor)	-0.4587
(BrainTumor) -> (G3196A)	0.2076

Table 2.28.
Pearson correlation between selected variables based on the influence scores for female samples.

		T3197C	A10006G	A1555G	A10398G	G9952A	mcd status
BrainTumor		-.850**	-.856**	-.677**	-.733**	.469*	-.946**
	Sig.	0	0	0	0	0.016	0
mtOXDamageDbyA10006G		.490*	.520**	0.29	.623**	-0.07	.583**
	Sig.	0.011	0.007	0.15	0.001	0.736	0.002
CopyNumbyG3196A		0.03	-0.123	0.304	-0.284	-.463*	0.129
	Sig.	0.885	0.55	0.131	0.16	0.017	0.53
mtOXDamageDbyCopyNum		-0.277	-.467*	0.043	-.418*	-0.27	-0.201
	Sig.	0.171	0.016	0.834	0.034	0.182	0.325
mtOXDamageD		0.19	0.081	0.089	0.359	0.015	0.228
	Sig.	0.354	0.695	0.664	0.072	0.943	0.262
mtOXDamage		0.166	0.231	-0.055	0.385	0.132	0.198
	Sig.	0.417	0.256	0.79	0.052	0.521	0.331
CopyNum		-0.336	-.462*	0.009	-.539**	-0.253	-0.288
	Sig.	0.093	0.018	0.965	0.004	0.212	0.153

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

Table 2.28. (cont.)
Pearson correlation between selected variables based on the influence scores for female samples.

		mtOX DamageDby A10006G	CopyNumby G3196A	mtOX DamageDby CopyNum	mtOX DamageD	mtOX Damage	CopyNum	copyno
BrainTumor		-.639**	-0.035	0.291	-0.296	-0.286	.396*	0.195
	Sig.	0	0.865	0.149	0.142	0.157	0.045	0.34
mtOXDamageD by A10006G		1	-0.228	-0.113	.894**	.786**	-.544**	-0.369
	Sig.		0.262	0.582	0	0	0.004	0.064
CopyNumby G3196A		-0.228	1	.835**	-0.202	-.468*	.879**	.493*
	Sig.	0.262		0	0.323	0.016	0	0.011
mtOXDamageD byCopyNum		-0.113	.835**	1	0.113	-0.216	.863**	.426*
	Sig.	0.582	0		0.582	0.29	0	0.03
mtOXDamageD		.894**	-0.202	0.113	1	.796**	-.392*	-0.311
	Sig.	0	0.323	0.582		0	0.048	0.123
mtOXDamage		.786**	-.468*	-0.216	.796**	1	-.610**	-0.344
	Sig.	0	0.016	0.29	0		0.001	0.085
CopyNum		-.544**	.879**	.863**	-.392*	-.610**	1	.577**
	Sig.	0.004	0	0	0.048	0.001		0.002

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

Chapter V

CONCLUSIONS

This study revealed the following major findings:

1. Relative mtDNA copy number were higher in female intractable epilepsy patients compared to non-epileptic control samples (p=0.01).
2. Oxidative mtDNA damage was lower in female epileptics compared to non-epileptic control samples (p=0.24), and lower in MCD compared to non-MCD (p=0.58).
3. The frequency of mtDNA SNP G9952A was higher in female epilepsy samples compared to controls.
4. Bayesian network showed significant relationships ($p < 0.05$) between epilepsy, mcd, oxidative mtDNA damage, mtDNA copy number, and G9952A.
5. Relative mtDNA copy number were higher in female neoplastic intractable epilepsy patients compared to non-epileptic control samples (p=0.34).
6. Oxidative mtDNA damage was higher in female neoplastic epileptic compared to control samples (p=0.74), and no differences in MCD epilepsy patients compared to non-MCD (p=0.99).
7. The frequency of mtDNA G9952A was found in higher frequencies in neoplastic epilepsy samples compared to controls.

8. Female neoplastic epilepsy phenotypes were predicted by G9952A (OR= 9.91E9, 95% CI [0, NaN]), level of relative mtDNA copy number (4.10, 95% CI [0.28, 60.18]), and level of oxidative mtDNA damage (OR= 2.34, 95% CI [0.20, 27.21])
9. Bayesian network showed relationships ($p < 0.05$) between brain tumor with G9952A, A1555G, T3197C, A10006G, A10398G, oxidative mtDNA damage with relative mtDNA copy number, and brain tumor with relative mtDNA copy number in female samples.
10. These data suggest that mitochondria play a critical role in the development of both epilepsy and brain tumor.

Directions for future research

The results of the present study are intended to inspire research regarding the possible roles of mitochondrial dysfunction in MCD intractable epilepsy. The data show that changes in mtDNA copy number, oxidative mtDNA damage, and function of COIII in the mitochondria as areas of interest for research in MCD intractable epilepsy. Our results suggest that changes in mtDNA copy number may depend on the levels of the environmental insult that result in changes in metabolic activity and production of ROS in the mitochondria. Thus, more studies exploring and elucidating the role of mitochondrial proliferation as a compensatory response to oxidative stress are needed. Hence, a study which measures, in addition to the mtSNPs, oxidative mtDNA damage and relative mtDNA copy number, the expression of both nuclear and mitochondrial genes and proteins involved in mitochondrial biogenesis, replication, mitochondrial base

excision repair and antioxidant response elements in response to oxidative damage is needed.

Since a trend that both MCD and non-MCD samples, as well as, female epilepsy samples were predicted by levels of mtDNA copy number and the mtSNP: G9952A, our results indicate that research regarding mitochondrial dysfunction mediated by COIII activities and increase in mtDNA content in epileptogenesis in MCD is greatly needed. Lastly, since the majority of the MCD patients are classified as malformations due to abnormal glial and neuronal proliferation in the epilepsy patients, research regarding the environmental exposures in this stage of brain development and epileptogenesis is warranted. Since mitochondrial gene expression and regulation are becoming increasingly relevant to human diseases determination of the potential signaling pathways involved in mtDNA copy number replication and regulation will help us to develop new approaches to maintaining healthy mitochondria and preventing epileptogenesis.

LIST OF REFERENCES

Aarts, M.M., and Tymianski, M. (2003). Novel treatment of excitotoxicity: targeted disruption of intracellular signaling from glutamate receptors. *Biochemical Pharmacology, 66,* 877-886.

Abu-Amero, K.K., Morales, J., Mohamed, G.H., et al. (2006). The role of mitochondrial haplogroups in glaucoma: a study in an Arab population. *Molecular Vision, 14,* 518-522.

Alexander, G.M., and Godwin, D.W. (2006). Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy Research, 71,* 1-22.

Allen, K.M., and Walsh, C.A. (1999). Genes that regulate neuronal migration in the cerebral cortex. *Epilepsy Research,* 36, 143–54.

Anderson, J.K. (2004). Oxidative stress in neurodegeneration: cause or consequence. *Nature Reviews Neuroscience, 5,* S18-S25.

Andre, V., Flores-Hernandez, J., Cepeda, C., Starling, A.J., Nguyen, S. et al. (2004). NMDA receptor alterations in neurons from pediatric cortical dysplasia tissue. *Cerebral Cortex, 14,* 634-646.

Aronica, E., Gorter, J.A., Jansen, G.H., van Veelen, C.W.M., Rijen, P.C., et al. (2003). Expression and cell distribution of group I and group II metabotropic glutamate receptor subtypes in taylor-type focal cortical dysplasia. *Epilepsia, 44,* 785-795.

Arundine, M., and Tymianski, M. (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium, 34,* 325-337.

Astridinis, A., Senapedis, W., Coleman, T.R., and Henske, E.P. (2003). Cell cycle-regulated phosphorylation of hamartin, the product of the tuberous sclerosis complex 1 gene, by cyclin-dependent kinase 1/cyclin B. *The Journal of Biological Chemistry, 278,* 51372-51379.

Ayala-Torres, S., Chen, Y., Svoboda, T., et al. (2000). Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods, 22,* 135-147.

Babb, T.L., Ying, Z., Mikuni, N., Nishiyama, K., Drazba, J.,et al. (2000). Brain plasticity and cellular mechanisms of epileptogenesis in human and experimental cortical dysplasia. *Epilepsia, 41,* S76-S81.

Bai, R., Perng, C., Hsu, C. (2004). Quantitative PCR analysis of mitochondrial DNA content in patients with mitochondrial disease. *Annals New York Academy of Sciences. 1011,* 304-309.

Bai, R., Leal, S.M., Covarrubias, D. et al. (2007). Mitochondrial genetic background modifies breast cancer risk. *Cancer Research, 67,* 4687-4694.

Bai, R., Wong, L., and Leal, S.M. (2008). Mitochondrial DNA variant interactions modify breast cancer risk. *Journal of Human Genetics, 53,* 924-928.

Baraban, S.C. (2001). Epileptogenesis in the dysplastic brain: A revival of familiar themes. *Epilepsy Currents, 1,* 6-11.

Barkovich, A.J., Kuzniecky, R.I., Jackson, G.D., et al. (2005). A developmental and genetic classification for malformations of cortical development. *Neurology, 65,* 1873-1887.

Baron, M., Kudin, A., and Kunz, W. (2007). Mitochondrial dysfunction in neurodegenerative disorders. *Biochemical Society Transactions, 35,* 1228-1231.

Bast, T., Ramantani, G., Seitz, A., and Rating, D. (2006). Focal cortical dysplasia: Prevalence, clinical presentation and epilepsy in children and adults. *Acta Neurologica Scandinavica, 113,* 72-81.

Baybis, M., Yu, J., Lee, A., Golden, J.A., Weiner, H., et al. (2004). mTOR cascade activation distinguishes tubers from cortical dysplasia. *Annals of Neurology, 56,* 478-487.

Becker, A.J., Chen, J., Sebastian, P., Sabine, N., Beck, H., et al. (2002). Transcriptional profiling in human epilepsy: expression array and single cell real-time qRT-PCR analysis reveal distinct cellular gene regulation. *Molecular Neuroscience, 13,* 1327-1333.

Becker, A.J., Urbach, H., Scheffler, B., Baden, T., Normann, S., et al. (2002). Focal cortical dysplasia of taylor's balloon cell type: Mutational analysis of the TSC1 gene indicates a pathogenic relationship to tuberous sclerosis. *Annals of Neurology, 52,* 29-37.

Becker, E.B., and Bonni, A. (2006). Pin1 mediates neural-specific activation of the mitochondrial apoptotic machinery. *Neuron, 49,* 655-662.

Beleza, P. (2009). Refractory epilepsy: A clinical oriented review. *European Neurology, 62,* 65-71.

Benardete, E.A., and Kriegsteins, A. R. (2002). Increased excitability and decreased sensitivity to GABA in an animal model of dysplastic cortex. *Epilepsia, 43,* 970-982.

Bonin, M., Poths, S., Osaka, H. Wang, Y., Wada, K., et al. (2004). Microarray expression analysis of gad mice implicates involvement of Parkinson's disease associated UCH-Li in multiple metabolic pathways. *Molecular Brain Research, 126,* 88-97.

Brahimi-Horn, M., Bellot, G., and Pouysegur, J. (2011). Hypoxi and energetic tumor metabolism. *Current Opinion in Genetics and Development, 21,* 67-72.

Brinckmann, A., Weiss, C., Wilbert, F., et al. (2010). Regionalized pathology correlates with augmentations of mtDNA copy numbers in patients with myoclonic epilepsy with ragged-red fibers (MERRF-Syndrome). *PLoS ONE, 5,* e13513.

Buonocore, G., Perrone, S., and Bracci, R. (2001). Free radicals and brain damage in the newborn. *Biology of the Neonate, 79,* 180-186.

Cepeda, C., Andre, V., Levine, M.S., Salamon, N., Miyata, H., et al. (2006). Epileptogenesis in Pediatric cortical dysplasia: The dysmature cerebral developmental hypothesis. *Epilepsy & Behavior, 9,* 219-235.

Cepeda, C., Andre, V.M., Wu, N., Yamazaki, I., Uzgil, B., et al. (2007). Immature neurons and GABA networks may contribute to epileptogenesis in pediatric cortical dysplasia. *Epilepsia, 48,* 79-85.

Chang, E.F., Christie, C., Sullivan, J.E., et al. (2010). Seizure control outcomes after resection of dyembryoplastic neuroepithelial tumor in 50 patients. *Journal of Neurosurgy: Pediatrics, 5,* 123-130.

Charrier-Savournin, F.B., Chateau, M., Gire, V., Sedivy, J., Piette, J., and Dulic, V. (2004). P-21 mediated nuclear retention of cyclin B1-Cdk1 response to genotoxic stress. *Molecular Biology of the Cell, 15,* 3965-3976.

Chen, S., Chang, A.Y., and Chuang, Y. (2010). The potential role of mitochondrial dysfunctions in seizure-associated cell death in the hippocampus and epileptogenesis. *Journal of Bioenergetics and Biomembrane, 42,* 461-465.

Chen, X., and Sullivan, P.F. (2003). Single nucleotide polymorphism genotyping: biochemistry, protocol, cost, and throughput. *The Pharmacogenomics Journal, 3,* 77-96.

Chen, C., Wu, Y., Cheng, M., et al. (2007). Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. *Biochemical and Biophysical Research Communications, 359,* 335-340.

Chen, T., He, J., Shen, L., et al. (2011). The mitochondrial DNA 4,977-bp deletion and its implication in copy number alteration in colorectal cancer. *BMC. Medical Genetics, 12,* 8-16

Chevassus-au-Louis, N., Ben-Ari, Y., and Vergnes, M. (1998). Decreased seizure threshold and more rapid rate of kindling in rats with cortical malformations induced by prenatal treatment with methylazoxymethanol. *Brain Research, 812,* 252-255.

Chevassus-au-Louis, N., Baraban, S.C., Gaiarsa, J.L., and Ben-Ari, Y. (1999). Cortical malformations and epilepsy: New insights from animal models. *Epilepsia, 40,* 811-821.

Choi, I., Cho, J., Lee, M., and Choi, B. (2005). Pilcarpine-induced seizure susceptibility in rats following prenatal methylazoxymethanol treatment. *Biological and Pharmaceutical Bulletin., 28,* 1408-1413.

Chuang, Y. (2010). Mitochondrial dysfunction and oxidative stress in seizure-induced neuronal death. *Acta Neurologica Taiwanica, 19,* 3-15.

Cordon-Cardo., C. (1995). Mutation of cell cycle regulators. *American Journal of Pathology, 147,* 545-560.

Crino, P.B., Trojanoswski, J.Q., Dichter, M.A., and Eberwine, J. (1996). Embryonic neuronal markers in tuberous sclerosis: Single-cell molecular pathology. *Proceedings of the National Academy of Sciences of the United States of America,, 93,* 14152-14157.

Crino, P.B., Miyata, H., and Vinters, H.V. (2002). Neurodevelopmental disorders as a cause of seizures: Neuropathologic, genetic, and mechanistic considerations. *Brain Pathology, 12,* 212-233.

Crino, P.B. (2004). Malformations of cortical development: Molecular pathogenesis and Experimental strategies. (pp 175-191). In D. Binder (Ed.) Recent Advances in Epilepsy Research. Advances in Experimental Medicine and Biology. Georgetown, TX: Plenum Publishers.

Croll, S.D., Goodman, J.H., and Scharfman, H.E. (2004). Vascular endothelial growth factor (VEGF) in seizures: A double-edged sword. (pp 57-65). In D. Binder (Ed.) Recent Advances in Epilepsy Research. Advances in Experimental Medicine and Biology. Georgetown, TX: Plenum Publishers.

Crompton, M. (2000). Mitochondrial intermembrane junctional complexes and their role is cell death. *Journal of Physiology, 529,* 11-21.

Danbolt, N.C. (2001). Glutamate uptake. *Progress in Neurobiology, 65,* 1-105.

Deb, P., Sharma, M.C., Tripathi, M., Chandra, S., Gupta, A. et al. (2006). Expression of CD34 as a novel marker for glioneuronal lesions associated with chronic intractable epilepsy. *Neuropathology and Applied Neurobiology, 32,* 461-468.

De la Monte, S.M., Luong, T., Neely, T.R., et al. (2000). Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's Disease. *Laboratory Investigation, 80,* 1323-1335.

DeLorenzo, R.J., Pal, S., and Sombati, S. (1998). Prolonged activation of the N-methyl-D-aspartate receptor-Ca2+ transduction pathway causes spontaneous recurrent epileptiform discharges in hippocampal neurons is culture. *Neurobiology, 95*, 14482-14487.

Devi, P.U., Monocha, A., and Vohora, D. (2008). Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers. *Expert Opinion on Pharmacotherapy, 9,* 3169-3177.

DiMauro, S. (2007). Mitochondrial DNA medicine. *Bioscience Reports, 27,* 5-9.

D'Incerti, L. (2003). Morphological neuroimaging of malformations of cortical development. *Epileptic Disorders, Suppl 2,* S9-S66.

Donner, E.J., and Snead, O.C. (2006). New generation anticonvulsants for the treatments of epilepsy in children. The Journal of the American Society for Experimental *NeuroTherapeutics, 3*, 170-180.

Edwards, J. (2009). Quantification of mitochondrial DNA (mtDNA) damage and error rates by real-time PCR. *Mitochondrion, 9,* 31-35.

Englund, C., Fokerth, R.D., Born, D., Lacy, J.M., and Hevner, R.F. (2005). Aberrant neuronal-glial differentiation in taylor-type focal cortical dysplasia (typeIIA/B). *Acta Neuropathologia, 109,* 519-533.

Fantel, A.G., Mackler, B., Stamps, L.D., Tran, T.T., and Person, R.E. (1998). Reactive oxygen species and DNA oxidation in fetal rat tissues. *Free Radical Biology & Medicine, 25,* 95-103.

Fassunke, J., Blumcke, I., Lahl, R., Elger, C.E., Schramm, J., et al. (2004). Analysis of chromosomal instability in focal cortical dysplasia of Taylor's balloon cell type. *Acta Neurologica, 108,* 129-134.

Fauser, S., Huppertz, H., Bast, T., Strobi, K., Pantazis, G. (2006). Clinical characteristics in focal cortical dysplasia: a retrospective evaluation in a series of 120 patients. *Brain, 129,* 1907-1916.

Ferraguti, F., and Shigemoto, R. (2006). Metabotropic glutamate receptors. *Cell & Tissue Research,* 326, 483-504.

Filiano, J.J., Goldenthal, M.J., Rhodes, H., et al. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. *Journal of Child Neurology, 17,* 435-439.

Fishel, M.L., Vasko, M.R., and Kelley, M.R. (2007). DNA repair in neurons: So if they don't divide what's to repair? *Mutation Research, 614,* 24-36.

Fisher, T.L., and White, M.F. (2004). Signaling Pathways: The benefits of good communication. *Current Biology, 14,* R1005-R1007.

Fukuda, M., Yamauchi, H., Yamamoto, H., et al. (2008). The evaluation of oxidative DNA damage in children with brain damage using 8-hydroxyguanosine levels. *Brain and Development, 30,* 131-136.

Geurts, J.J., Wolswik, G., Bo, L., van der Valk, P., Polman, C.H., et al. (2003). Altered expression patterns of group I and II metabotropic receptors in multiple sclerosis. *Brain, 126,* 1755-1766.

Govori, V., Humolli, I., Cepreganov, M., et al. (2010). Brain tumors and epilepsy. *Acta Clinica Croatica, 49,* 133-138.

Gressens, P. (2000). Mechanisms and disturbances of neuronal migration. *Pediatric Research, 48,* 725-730.

Gu, G., Varoqueaux, F., and Simerly, R.B. (1999). Hormonal regulation of glutamate receptor gene expression in the anteroventral periventricular nucleus of the hypothalamus. *The Journal of Neuroscience, 19,* 3213-3222.

Guerrini, R., Sicca, F., and Parmeggiani, L. (2003). Epilepsy and malformations of the cerebral cortex. *Epileptic Disorders, 5* (Suppl 2), S9-S26.

Hader, W.J., Mackay, M., Otsubo, H., Chitoku, S., Weiss, S., et al. (2004). Cortical dysplastic lesions in children with intractable epilepsy: role of complete resection. *Journal of Neurosurgery: Pediatrics, 100,* 110-117.

Hallene, K.L., Oby, E., Lee, B.J., Santaguida, S., Bassanini, S., et al. (2006). Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience, 142,* 267-283.

Hanna, M., Nelson, P., Rahman, S., et al. (1996). Cytochrome c oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. *American Journal of Genetics, 63,* 29-36.

Hannan, A.J., Servotte, S., Katsnelson, A., Sisdiya, S., Blakemore, C., et al. (1999). Characterization of nodular neuronal heterotopia in children. *Brain, 122,* 219-238.

Harrington, E.P., Moddel, G., Najm, I.M., and Baraban, S.C. (2007). Altered glutamate receptor-transporter expression and spontaneous seizures in rats exposed to methylazoxymaethanol in utero. *Epilepsia, 48,* 158-168.

Harrison, J.F., Hollensworth, S.B., Spitz, D.R., Copeland, W.C., Wilson, G.L., and LeDoux, S.P. (2005). Oxidative stress-induced apoptosis in neurons correlates with mitochondrial DNA base excision repair pathway imbalance. *Nucleic Acids Research, 33,* 4660-4671.

Hengstschlager, M., and Rosner, M. (2003). The cell cycle and tuberous sclerosis. *Progress in Cell Cycle Progress, 5,* 43-48.

Higushi, M. (2007). Regulation of mitochondrial DNA content and cancer. *Mitochondrion. 7,* 53-57

Hosgood III, H., Liu, C., Rothman, D. et al. (2010). Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. *Carcinogenesis, 31,* 847-849.

Howell, N., Oostra, R., Bolhuis, P., et al. (2003). Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber Hereditary Optic Neuropathy. *American Journal of Genetics, 72,* 1460-1469.

Hua, Y., and Crino, P.B. (2003). Single cell lineage analysis in human focal cortical dysplasia. *Cerebral Cortex, 13,* 693-699.

Ito, N., and Rubin, G.M. (1999). Gigas, a *Drosophila* homolog of tuberous sclerosis gene product-2, reglates the cell cycle. *Cell, 96,* 529-539.

Jarrett, S.G., Liang, L.P., Hellier, J.L., Staley, K.J., and Patel, M. (2008). Mitochondrial DNA damage and impaired base excision repair during epileptogenesis . *Neurobiology Disease, 30,* 130-138.

Jeng, J., Yeh, T., Lee, J., et al. (2008). Maintenance of mitochondrial DNA copy number and expression are essential for preservation of mitochondrial function and cell growth. *Journal of Cellular Biochemistry, 103,* 347-357.

Jiang, W., Masayesva, B., Zahurak, M., et al. (2005). Increased mitochondrial DNA content in saliva associated with head and neck cancer. *Human Cancer Biology, 11,* 2486-2491.

Kasai, H. (1997). Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutation Research, 387,* 147-163.

Katayama, Y., Maeda, K., Lizuka, T., et al. (2009). Accumulation of oxidative stress around the stroke-like lesions of MELAS patients. *Mitochondrion, 9,* 306-311.

Kim, M. M., Clinger, B.G., Ha, P.K., et al. (2004). Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clinical Cancer Research, 10,* 8512-8515.

Kisby, G.E., Olives, A., Standley, M., et al. (2006). Genotoxicants target distinct molecular networks in neonatal neurons. *Environmental Health Perspectives 114***,** 1703-1712.

Klein, J.A., and Ackerman, S.L. (2003). Oxidative stress, cell cycle, and neurodegeneration. *Journal of Clinical Investigation, 111,* 785-793.

Klug, W.S., and Cummings, M.R. (1997). Concepts of Genetics. 5th Edition. New Jersey: Prentice Hall.

Konoshi, Y., and Bonni, A. (2003). The E2F-Cdc2 cell-cycle pathway specifically mediates activity deprivation-induced apoptosis of postmitotic neurons. *The Journal of Neuroscience, 23,* 1649-1658.

Krauss, G. (2003). Biochemistry of signal transduction and regulation. 3rd Edition. WILEY-VCH GmbH & Co. KGaA.

Kudin, A., Zsurka, G., Elger, C., et al. (2009). Mitochondrial involvement in temporal lobe epilepsy. *Experimental Neurobiology, 218,* 326-332.

Kunz, W.S. (2002). The role of mitochondria in epileptogenesis. *Seizure Disorders, 15,* 179-184.

Kunz, W.S., Goussakov, I.V., Beck, H., and Elger, C.E. (1999). Altered mitochondrial oxidative phosphorylation in hippocampal slices of kainite- treated rats. *Brain Research, 826*, 236-242.

Kuzniecky, R.I., and Barkovich, A. J. (2001). Malformations of cortical development and epilepsy. *Brain and Development, 23,* 2-11.

Kuzniecky, R.I., and Jackson, G.D. (2008). Malformatoin of cortical development. In J. Engel, T.A. Pedley, and J. Aicardi, Epilepsy: A comprehensive textbook, volume 1(pp. 2575-2630) , Philadelphia, PA. Lippincott Williams & Wilkins.

Kwok, P. (2001). Methods for genotyping single nucleotide polymorphisms. *Annual Review of Genomics and Human Genetics, 2,* 235-258.

Lamb, R.F., Roy, C., Difenbach, T.J., Vinters, H.V., Johnson, M.W., et al. (2000). The TSC1 tumor suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho. *Nature Cell Biology, 2,* 281-287.

Lan, Q., Lim, U., Liu, C. et al. (2008). A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Neoplasia, 112,* 4247-4249.

Langley, B., and Ratan, R.R. (2004). Oxidative stress-induced death in the nervous system: cell cycle dependent or independent? *Journal of Neuroscience Research, 77,* 621-629.

LeGac, G., Esteve, P., Ferec, C., and Pradhan, S. (2006). DNA Damage-induced down-regulation of human Cdc25C and Cdc2 is mediated by cooperation between p53 and maintenance DNA (cytosine-5) methyltransferase 1. *The Journal of Biological Chemistry, 281,* 24161-24170.

Lee, H., Yin, P., Lu, Ching, et al. (2000). Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical Journal, 348,* 425-432.

Lee, H., and Wei, Y. (2005). Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *The International Journal of Biochemistry and Cell Biology, 37,* 822-834.

Lee, J., Park, K., Im, J., et al. (2010). Mitochondrial DNA copy number in peripheral blood associated with cognitive function in apparently healthy elderly women. *Clinica Chimica Act, 411,* 592-596.

Lenroot, R., and Giedd, J. (2006). Brain development in children and adolescents: Insights from anoatomical magnetic resonance imaging. *Neuroscience and Biobehavioral Reviews, 30,* 718-729.

Lewis, W., Day, B.J., Kohler, J.J., et al. (2007). Decreased mtDNA, oxidative stress, cardiomyopathy and death from transgenic cardiac targeted human mutant polymerase γ. *Laboratory Investigation. 87,* 326-335.

Liang, B.C., and Hays, L., (1996). Mitochondrial DNA copy number changes in human gliomas. *Cancer Letters, 106,* 167-173.

Lin, C., Wang, L., Tsai, C., et al. (2008). Low copy number and low oxidative damage of mitochondrial DNA are associated with tumor progression in lung cancer tissues after neoadjuvant chemotherapy. *Interactive Cardiovascular and Thoracic Surgery,7,* 954-958.

Liu, C., Lee, C., Hong, C., and Wei, Y. (2004). Mitochondrial DNA mutation and depletion increase the susceptibility of human cells to apoptosis. *Annals New York Academy of Sciences, 1011,* 133-145.

Liu, C., Cheng, W., Lee, C. et al. (2006). Alterations in the copy number of mitochondrial DNA in leukocytes of patients with mitochondrial encephalomyopathies. *Acta Neurologica Scandinavia, 113,* 334-341.

Lopez, J., Gonzalez, M., Lorigados, L., et al. (2007). Oxidative stress in surgically treated patients with refractory epilepsy. *Clinical Biochemistry, 40,* 292-298.

Lortie, A., Plouin, P., Chiron, C., Delalande, O., and Dulac, O. (2002). Characteristics of epilepsy in focal cortical dysplasia in infancy. *Epilepsy Research, 51,* 133-145.

Kim, J., Koyama, R., Yamada, R.X., Yamada, M.K., et al. (2004). Environmental control of the survival and differentiation of dentate granule neurons. *Cerebral Cortex Disorder, 14,* 1358-1364.

Ma, Y., Chen, Y., Lu, C., Liu, C., and Wei, Y. (2005). Upregulation of matrix metalloproteinase 1 and disruption of mitochondrial network in skin fibroblasts of patients with MERRF syndrome. *Annals New York Academy of Sciences, 1042,* 55-63.

Malakhova, L., Bezlepkin, V., Antipova, V., et al. (2005). The increase in mitochondrial DNA copy number in the tissue of γ-irradiated mice. *Cellular and Molecular Biology Letter, 10,* 721-732.

Mambo, E., Chatterjee, A., Xing, M., et al. (2005). Tumor-specific changes in mtDNA content in human cancer. *International Journal of Cancer, 116,* 921-924.

Manent, J., and Represa, A. (2007). Neurotransmitters and brain maturations: Early paracrine actions of GABA and glutamate modulate neuronal migration. *The Neuroscientists,13,* 268-279.

Mao, L., and Wang, J.Q. (2002). Glutamate cascade to cAMP response element-binding protein phosphorylation in cultured striatal neurons through calcium-coupled group I metabotropic glutamate receptors. *Molecular Pharmacology, 62,* 473-484.

Markert, J.M., Fuller, C.M., Gillespie, G.Y., Bubien, J.K., McLean, L.A., et al. (2001). Differential gene expression profiling in human brain tumors. *Physiological Genomics, 5,* 21-33.

Mathern, G.W., Andres, M., Salamon, N., Chandra, P.S., Andre, V.M. et al. (2007). A hypothesis regarding the pathogenesis and epileptogenesis of pediatric cortical dysplasia and hemimegalencephaly based on MRI cerebral volumes of NeuN cortical cell densities. *Epilepsia, 48,* 74-78.

Mayevsky, A. (2009). Mitochondrial function and energy metabolism in cancer cells: Past overview and future perspectives. *Mitochondrion, 9,* 165-179.

Mefford, H.C., Muhle, H., Ostertag, P. et al. (2010). Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLos Genetics, 6*, n.p.

Myatt, S., Brosens, J., and Lam, E. (2011). Sense and sensibility: FOXO and ROS in cancer development and treatment. *Antioxidants and Redox Signaling, 14,* 675-687.

Migliore, L., and Coppede, F. (2002). Genetic and environmental factors in cancer and neurodegenerative diseases. *Mutation Research, 512,* 135-153.

Miller, L.D., Long, P.M., Mukherjee, S., McShane, L.M., and Liu, E.T. (2002). Optimal gene expression by microarrays. *Cancer Cell, 2,* 353-361.

Milolaza, A., Kubista, A., Rosner, M., and Hengstschlager, M. (2002). Evidence for separable functions of tuberous sclerosis gene products in mammalian cell cycle regulation. *Journal of Neuropathology and Experimental Neurology, 61,* 154-163.

Mischel, P.S., Nguyen, L.P., and Vinters, H.V. (1995). Cerebral cortical dysplasia associated with pediatric epilepsy. Review of neuropathologic features and proposal for a grading system. *Journal of Neuropathy and Experimental Neurology, 54,* 137-153.

Miyata, H., Chiang, A. C.Y., and Vinters, H.V. (2004). Insulin signaling pathways in cortical dysplasia and TSC-Tubers: Tissue microarray analysis. *Annals of Neurology, 56,* 510-519.

Moddel, G., Jacobson, B., Ying, Z., Janigro, D., Bingaman, W., et al. (2005). The NMDA receptor NR2B subunit contributes to epileptogenesis in human cortical dysplasia. *Brain Research, 1046,* 10-23.

Montenegro, M.A., Guerreiro, M.M., Lopes-Cendes, I., Guerreiro, C.A., and Cendes, F. (2002). Interrelationship of genetics and prenatal injury in the genesis of malformations of cortical development. *Archives of Neurology, 59,* 1147-1153.

Montier, L.L., Deng, J.J., and Bai, Y. (2009). Number matters: control of mammalian mitochondrial DNA copy. *Journal of Genetics and Genomics, 36,* 125-131.

Montenegro, M.A., Cendes, F., Lopes-Cendes, I., Guerreiro, C.A., Li. L.M., et al. (2007). The clinical spectrum of malformations of cortical development. *Arq Neurosiquiatr, 65,* 196-201.

Mulley, J.C., Scheffer, I.E., Harkin, L.A., Berkovic, S.F., and Dibbens, L.M. (2005). Susceptibility genes for complex epilepsy. *Human Molecular Genetics, 14,* R243-R249.

Munakata, M., Watanabe, M., Otsuki, T., Nakama, H., Arima, K., et al. (2007). Altered distribution of KCC2 in cortical dysplasia in patients with intractable epilepsy. *Epilepsia, 48,* 837-844.

Narayanan, V. (2003). Tubersous sclerosis is complex: genetics to pathogenesis. *Pediatric Neurology, 29,* 404-409.

Neder, L., Valente, V., Carlotti, C.G., Leite, J.P., Assirati, J.A., et al. (2002). Glutamate NMDA receptor subunit R1 and GAD mRNA expression in human temporal lobe epilepsy. *Cellular and Molecular Neurobiology, 22,* 689-699.

Niehusmann, P., Surges, R., von Wrede, R., et al. (2011). Mitochondrial dysfunction due to Leber's hereditary optic neuropathy as a cause of visual loss during assessment for epilepsy surgery. *Epilepsy and Behavior, 20,* 38-43.

Notenboom, R.G., Hampson, D.R., Jansen, G.H., van Rijen, P.C., van Veelen, C.W., et al. (2006). Up-regulation of hippocampal metabotropic glutamate receptor 5 in temporal lobe epilepsy patients. *Brain, 129,* 96-107.

Ottman, R. (1997). Genetic epidemiology of epilepsy. *Epidemiologic Reviews, 19,* 120-128.

Pakendorf, B., and Stoneking, M. (2005). Mitochondrial DNA and human evolution. *Annual Review of Genomics and Human Genetics, 6,* 165-183.

Palmini, A., Anderman, E., and Anderman, F. (1994). Prenatal events and genetic factors in epileptic patients with neuronal migration disorders. *Epilepsia, 35,* 965-973.

Paredes, M.F., and Baraban, S.C. (2002). A review of gene expression patterns in the malformed brain. *Molecular Neurobiology, 26,* 1-8.

Paredes, M., Pleasur, S.J., and Baraban, S.C. (2006). Embryonic and early postnatal abnormalities contributing to the development of hippocampal malformations in a rodent model of dysplasia. *Journal of Comprehensive Neurology, 495,* 133-148.

Park, S., Shin, M., Kim, H., et al. (2009). Alterations of mitochondrial DNA sequence and copy number in nasal polyp tissue. *Mitochondrion, 9,* 318-325

Patil, C., Ahire, Y., Pathade, P., et al. (2011). Free radical epilepsy and anti-oxidant: An overview. *International Research Journal of Pharmacy, 2,* 64-71.

Pawitan, Y., Michiels, S., Koscielny, S., Gusnanto, A., and Ploner, A. (2005). False discovery rate, sensitivity and sample size for microarray studies. *Bioinformatics, 21,* 3017-3024.

Penta, J., Johnson, F., Wachsman, J., et al. (2001). Mitochondrial DNA in human malignancy. *Mutation Research, 488,* 119-133.

Pettersson, M, Bylund, M., and Alderborn, A. (2003). Molecular haplotype determination using allele-specific PCR and pyrosequencing technology. *Genomics, 82,* 390-396.

Pfeifer, R.M., and Gail, M.H. (2003). Sample size calculations for population-and family-based case-control association studies on marker genotypes. *Genetic Epidemiology, 25,* 136-148.

Pieczenik, S., and Neustadt, J. (2007). Mitochondrial dysfunction and molecular pathways of disease. *Experimental and Molecular Pathology, 83,* 84-92.

Pillai, J.J., Hessler, R.B., Allison, J.D., Park, Y.D., Lee, M.R., et al. (2002). Advanced MR Imaging of cortical dysplasia with or without neoplasm: A report of two cases. *American Journal of Neuroradiology, 23,* 1686-1691.

Poduri, A., Chang, B.S., and Walsh, C.A. (2008). Epileptogenic cerebral cortical malformations. In J.M. Pellock. Pediatric epilepsy: diagnosis and therapy (pp. 101-116), New York, NY, Demos Medical Publishing.

Prasad, A.N., Prasad, C., and Stafstrom, C.E. (1999). Recent advances in the genetics of epilepsy: Insights from human and animal studies. *Epilepsia, 40,* 1329-1352.

Price D.J., and Willshaw, D.J. (2000). *Mechanisms of cortical development.* New York: Oxford University Press.

Radpour, R., Fan, A., Kohler, C., et al. (2009). Current understanding of mitochondrial DNA in breast cancer. *The Breast Journal, 15,* 505-509.

Raol, Y.H., Lynch, D.R., and Brooks-Kayal, A.R. (2001). Role of excitatory amino acids in developmental epilepsies. *Mental Retardation and Developmental Disabilities Research Reviews, 7,* 254-260.

Raven, P.H., and Johnson, G.B. (1996). *Biology.* 4th Edition. Boston: Wm. C. Brown Publishers.

Ravizza, T., Boer, K., Redeker, S., Spliet, W.G.M., van Rijen, P.C, et al. (2006). The IL-β system in epilepsy-associated malformations of cortical development. *Neurobiology of Disease, 24,* 128-143.

Reuter, S., Subash, C., Gupta, C., et al. (2010). Oxidative stress, inflammation, and cancer. How are they linked? *Free Radical Biology and Medicine, 49,* 1603-1616.

Rickert, C.H., and Paulus, W. (2001). Epidemiology of central nervous system tumors in childhood and adolescence based on the new WHO classification. *Child's Nervous System, 17,* 503-511.

Rickert, C.H. (2006). Cortical dysplasia: neuropathological aspects. *Child's Nervous System. 22,* 821-826.

Robertson, C.L. (2004). Mitochondrial dysfunction contributes to cell death following traumatic brain injury in adult and immature animals. *Journal of Bioenergetics and Biomembranes, 36,* 364-368.

Rodier, P.M. (1995). Developing brain as a target of toxicity. *Environmental Health Perspectives, 103 (S6),* 73-76.

Roma, V., and Ebner, F.F. (1999). Effectof enriched environment rearing of impairments in cortical excitability and plasticity after prenatal alcohol exposure. *The Journal of Neuroscience. 19,* 10993-11006.

Saneto., R.P., and Wyllie, E. (2000). Epilepsy surgery in infancy. *Seminars in Pediatric Neurology, 7,* 204-215.

Santos, J.H., Meyer, J.N., Mandavilli, B.S., and Van Houten, B. (2006). Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. *Methods in Molecular Biology, 314,* 183-199.

Sas, K., Robotka, H., Toldit, J., et al. (2007). Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *Journal of Neurological Sciences, 257,* 221-239.

Scher, M.S. (2003). Prenatal contributions to epilepsy: Lessons from the bedside. *Epileptic Disorders, 5,* 77-91.

Schick, V., Majores, M., Engels, G., Spitoni, S., Koch, A., et al. (2006). Activation of Akt Independent of PTEN and CTMP tumor-suppressor gene mutations in epilepsy-associated taylor-type focal cortical dysplasias. *Acta Nueropathologia, 112,* 715-725.

Schick, V., Majores, M., Koch, A., Elger, C.E. , Schramm, J. et al. (2007a). Alterations of phosphatidylinositol 3-kinase pathway components in epilepsy-associated glioneuronal lesions. *Epilepsia,48,* 65-73.

Schick, V., Majores, M., Fassunke, J., Engels, G., Simon, M., et al. (2007b). Mutational and expression analysis of CDK1, cyclin2A and cyclinB1 in epilepsy-associated glioneuronal lesions. *Neuropathology and Applied Neurobiology, 33,* 152-162.

Schwartzkroin, P.A., and Walsh, C.A. (2000). Cortical malformations and epilepsy. *Mental Retardation and Developmental Diagnosis, 6,* 268-280.

Sen, A, Martinian, L., Nikolic, M., Walter, M.C., Thom, M., et al. (2007). Increased NKCC1 expression in refractory human epilepsy. *Epilepsy Research, 74,* 220-227.

Shackelford, R.E., Kaufmann, W.K., and Paules, R.S. (1999). Cell cycle control, checkpoint mechanisms, and genotoxic stress. *Environmental Health Perspectives, 107,* 5-24.

Shah, N.S., Mitchell, W.G., and Boles, R.G. (2002). M itochondrial disorders: A potential under-recognized etiology of infantile spasms. *Journal of Child Neurology, 17,* 369-372.

Shah, O.J., and Hunter, T. (2005). Tuberous sclerosis and insulin resistance. *Cell Cycle, 41,* 46-51.

Shen, J., Platek, M., Mahasneh, A., et al. (2010). Mitochondrial copy number and risk of breast cancer: A pilot study. *Mitochondrion, 10,* 62-68.

Sherman, S.L. (1997). Evolving methods in genetic epidemiology. IV. Approaches to non-Mendelian inheritance. *Epidemiologic Reviews, 19,* 44-51.

Shinnar, S., Pellock, J.M. (2002). Prognosis of pediatric epilepsy. *Journal of child neurology, 17S1,* S4-17.

Shokolenko, I., Venediktova, N., Bockkareva, G.L., et al. (2009). Oxidative stress induces degeneration of mitochondrial DNA. *Nucleic Acids Research, 37,* 2539-2548.

Simon, R., Radmacher, M.D., and Dobbin, K. (2002). Design of studies using DNA microarrays. *Genetic Epidemiology, 23,* 21-36.

Simon, H. U., Haj-Yehia, A., and Levi-Schaffer, F. (2004). Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis, 5,* 415-418.

Sisodiya, S.M., Lin, W.R., Harding, B.N., Squier, M.V., and Thom, M. (2002). Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. *Brain, 125,* 22-31.

Sisodiya, S.M., Thom, M., Thom, M., Lin, W., Bajaj, N.P.S. (2002). Abnormal expression of cdk5 in focal cortical dysplasia in humans. *Neuroscience Letters, 328,* 217-220.

Sisodiya, S.M., Martinian, L., Scheffer, G.L., van der Valk, P., Cross, J.H., et al. (2003). Major vault protein, a marker of drug resistance, is upregulated in refractory epilepsy. *Epilepsia, 44,* 1388-1396.

Sisodiya, S.M. (2004). Surgery for focal cortical dysplasia. *Brain, 127,* 2383-2384.

Smeets, V.M.J., van Lierop, B.A.G., Vanhoutvin, J.P.G., Aldenkamp, A.P., and Nijhuis, F.J.N. (2007). Epilepsy and employment: Literature Review. Epilepsy & Behavior, 10, 356-362.

Soucek, T., Rosner, M., Miloloza, A., Kubista, M., Cheadle, J.P., et al. (2001). Tuberous sclerosis causing mutants of the TSC2 gene product affect proliferation and p27 expression. *Oncogene, 20,* 4904-4909.

Spalice, A., Ruggieri, M., Grosso, S., et al. (2010). Dysembryoplastic neuronal tumors: A prospective clinicopathologic and outcome study of 13 children. *Pediatric Neurology, 43,* 395-402.

Statstrom, C.E., Lynch, M., and Sutula, T. (2000). Consequences of epilepsy in the developing brain: Implications for surgical management. *Seminar in Pediatric Neurology, 7,* 147-157.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J.M., Rhee, J. et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127,* 397-408.

Su, S., Jou, S., Cheng, W., et al. (2010). Mitochondrial DNA damage in spinal and bulbar muscular atrophy patients and carriers. *Clinica Chimica Acta, 411,* 626-630.

Sugimoto, T., Mori, C., Takanami, T., Sasagawa, Y., Saito, R., et al. (2008). Caenorhabditis elegans par21/mtssb-1 is essential for mitochondrial DNA replication and its defects causes comprehensive transcriptional alterations including a hypoxia response. *Experimental Cell Research, 314,* 103-114.

Sun, W., Wright, F., Tang, Z., et al. (2009). Integrated study of copy number states and genotype calls using high-density SNP arrays. *Nucleic Acids Research, 37,* 5365-5377.

Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., and Mihara, K. (2007). Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *The Journal of Biological Chemistry, 282,* 11521-11429.

Takahashi, D.K., Dinday, M.T., Barbaro, N.M., and Baraban, S.C. (2004). Abnormal cortical cells and astrocytomas in the Eker rat model of tuberous sclerosis complex. *Epilepsia, 45,* 1525-1530.

Tanaka, N., Goto, Y., Akanuma, J. et al. (2010). Mitochondrial DNA variants in a Japanese population of patients with Alzheimer's disease. *Mitochondrion, 10,* 32-37.

Theodore, W.H., Spencer, S.S., Wiebe, S., Langfit, S.T., Ali, A., et al. (2006). Epilepsy in North America: A report prepared under the auspices of the Global Campaign against Epilepsy, the International Bureau for Epilepsy, the International League Against Epilepsy, and the World Health Organization. *Epilepsia, 47*, 1700-1722.

Thom, M., Martinian, L., Sen,A., Cross, J.H., Harding, B.N., et al. (2005). Cortical neuronal densities and lamination in focal cortical dysplasia. *Acta Neuropathologia, 110,* 383-392.

Troost, B.K., Spliet, W.G., van Rijen, P.C., Gorter, J.A., and Aronica, E. (2008). Cellular distribution of vascular endothelial growth factor A (VEGFA) and B (VEGFB) and VEGF receptors 1 and 2 in focal cortical dysplasia type IIB. *Acta Neuropathologia*, *115*, 683-696.

Tsutsui, H., Kinugwa, S., and Matsushima, S. (2009). Mitochondrial oxidative stress and dysfunction in myocardial remodeling. *Cardiovasular Research, 81,* 449-456.

Tsutsui, H., Ide, T., Shiomi, T., et al. (2001). 8-Oxo-dGTPase, which prevents oxidative stress-induced DNA damage, increases in the mitochondrion from failing hearts. *Circulation, 104,* 2883-2885.

Tuppen, H.A., Blakely, E.L., Turnbull, D.M., et al. (2010). Mitochondrial DNA mutations and human disease. *Biochimica et Biophysica, 1797,* 113-128.

Tzen, C., Mau, B., and Hsu, H. (2007). Analysis of disease-associated ND4 mutations: How do we know which mutation is pathogenic. *Mitochondrion, 7,* 151-156.

Ueno, M., Katayama, K., Yamauchi, H., Nakayama, H., and Doi, K. (2006). Cell cycle and cell death regulation of neural progenitor cells in the 5-azacytidine (3AzC)-treated developing fetal brain*, Experimental Neurology, 198,* 154-166.

Umetsu, K., and Yuasa, I. (2005). Recent progress in mitochondrial DNA analysis. *Legal Medicine, 7,* 259-262.

Van De Graff, K. (2000). Human anatomy. 5th Edition. New York, NY: McGraw Hill.

Waldbaum, S., and Patel, M. (2010a). Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy. *Journal of Bioenergetics and Biomembranes, 42,* 449-455.

Waldbaum, S., and Patel, M. (2010b). Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Research, 88,* 22-45.

Wallace, D., Brown, M., and Lott, M. (1999). Mitochondrial DNA variation in human evolution and disease. *Gene, 238,* 211-230.

Wang, C., Li, Z., Lu, Y., Du, R., Katiyar, S., et al. (2006). Cyclin D1 repression of the nuclear respiratory factor 1 integrates nuclear DNA synthesis and mitochondrial function. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 11567-11572.

Wang, V.Y., Chang, E.F., Barbaro, N.M. (2006). Focal cortical dysplasia: a review of pathological features, genetics, and surgical outcome. *Neurosurgery Focus, 20,* 1-7.

Wang, Y., Liu, V., Xue, W., et al. (2005). The increase of mitochondrial DNA content in endometrial adenocarcinoma cells: A quantitative study using laser-captured microdissected tissues. *Gyencologic Oncology, 98,* 104-110.

Watanabe, M., Nonaka, R., Hagino, Y., and Kodama, Y. (1998). Effects of prenatal methylzaxymethanol treatment on striatal dopaminergic systems in rat brain. *Neuroscience Research, 30,* 135-144.

Wataya-Kaneda, M., Kaneda, Y., Hino, O., Adachi, H, Hirayama, Y., Seyama, K., et al. (2001). Cells derived from tuberous sclerosis show a prolonged S phase of the cell cycle and increased apoptosis. *Archives Dermatological Research, 293,* 460-469.

Waterham, H.R., Koster, J., van Roermund, C.W., Mooyer, P.A., Wanders, R.J., et al. (2007). A lethal defect of mitochondrial and peroxisomal fission. *New England Journal of Medicine, 356,* 1736-1741.

Wei, Y. (1998). Oxidative stress and mitochondrial DNA mutations in human aging. *Experimental Biology and Medicine, 217,* 53-63.

Wei, Y., Lee, C., Lee, H., et al. (2001). Increases of mitochondrial mass and mitiochondrial genome in association with enhanced oxidative stress in human cells harbouring 4,977 bp-deleted mitochondrial DNA. *Annals of New York Academy of Sciences, 928,* 97-112.

Wei, Y., and Lee, H. (2002). Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Experimental Biology and Medicine, 227,* 671-682.

Willmore, L.J., and Ueda, Y. (2002). Genetics of epilepsy. *Journal of Child Neurology, 17,* 18-27.

Wiseman, H., and Halliwell, B. (1995). Damage to DNA by reactive oxygen species and nitrogen species role in inflammatory disease and progression to cancer. *Biochemical Journal, 313,* 17-29.

Wong, M. (2007). Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia, 48,* 617-630.

Wong, M. (2008) Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia, 49,* 8-21.

Wong, M. (2009). Animal models of focal cortical dysplasia and tuberous sclerosis complex: recent progress towards clinical applications. *Epilepsia, 50 suppl.9,* 34-44.

Wosik, K., Ruffini, F., Almazan, G., Olivera, A., Nalbantoglu, J.L. et al. (2004). Resistance of human adult oligodendrocytes to AMPA/kainite receptor-mediated glutamate injury. *Brain, 127,* 2636-2648.

Wu, S., Ma, Y., Wu, Y., et al. (2010). Mitochondrial DNA mutation-elicited oxidative stress, oxidative damage, and altered gene expression in cultured cells of patients with MERRF syndrome. *Molecular Neurobiology, 41,* 256-266.

Yasin, S.A., Latak, K., Becherini, F., et al. (2010). Balloon cells in human cortical dysplasia and tuberous sclerosis: isolation of a pathological progenitor-like cell. *Acta Neuropathologica, 120,* 85-96.

Yin, P., Lee, H., Chau, G., et al. (2004). Alterations of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. *British Journal of Cancer, 90*, 2390-2396.

Yoshida, T., Tanaka., S., Mogi, A., Shitara, Y., and Kuwano, H. (2004). The clinical significance of cyclin b1 and wee1 expression in non-small-cell lung cancer. *Annals of Oncology, 15,* 252-256.

Zhai, Y., Geroge, C.A., Zhai, J., et al., (2003). Group II metabotropic glutamate receptor modulation of DOI-induced c-fos mRNA and excitatory response in the cerebral cortex. *Neuropsychopharmacology, 28*, 45-52.

Zhang, Q., Ding, L., Larson, D., et al. (2010). CMDS: a population-based method for identifying recurrent DNA copy number aberrations in cancer from high-resolution data. *Bioinformatics, 26,* 464-469.

Zheng, Y., Lofrredo, C.A., Alberg, A.J., Yu, J., Jones, R.T., et al. (2005). Less efficient G2-M checkpoint is associated with an increased risk in lung cancer in African Americans. *Cancer Research, 65,* 9566-9573.

Zsurka, G., Hample, K.G., Jardel, C., et al. (2010). Severe epilepsy as the major symptom of new mutations in the mitochondrial tRNA ^{Phe} gene. *Neurology, 74,* 507-512.

APPENDIX

Table A.1.
Clinical characteristics of the intractable epilepsy patients and controls

Sample	Age (years)	Gender	Initial Diagnosis	MCD Status	Pathology	Location in brain
1	3	M	intractable epilepsy	Non-MCD	new tumor dense gliosis	right frontal region
2	9	F	tumor	Non-MCD	pilocytic astrocytoma	post fossa, right cerebellar
3	14	M	intractable epilepsy	MCD	ganglioglioma	left temporal
4	8 ms	F	tumor	MCD	ganglioglioma	right frontal temporal
5	8	M	tumor	Non-MCD	oligodendroglioma	left parietal
6	12	F	tumor	Non-MCD	pilocytic astrocytoma	right posterior fossa
7	15	F	intractable epilepsy	MCD	cortical dysplasia, type Ia	right frontal temporal region
8	11	F	tumor	Non-MCD	pilocytic astrocytoma	right tectal
9	18	F	intractable epilepsy	MCD	focal cortical dysplasia, Taylor type IIB	right prefrontal cortex
10	6	M	intractable epilepsy	Non-MCD	mild neuronal disorganization	frontocentral cortex
11	13	F	intractable epilepsy	Non-MCD	cystic encephalomalacia	right parietal lobe
12	18	M	tumor	MCD	Dysembryoplastic Neuroepithelial Tumor (DNET)	right frontal
13	12	F	tumor	Non-MCD	glioblastoma multiforme	left temporal region
14	10	F	tumor	Non-MCD	medulloblastoma	posterior fossa
15	10	M	tumor	Non-MCD	pilocytic astrocytoma	left cerebellum
16	8 mos	M	tumor	Non-MCD	astrocytoma	right frontal temporal region
17	6	F	tumor	Non-MCD	choroid plexus papilloma	IV ventricle
18	3	F	intractable epilepsy	Non-MCD	meningioangiomatosis	right parietal tumor
19	5	M	tumor	Non-MCD	pilocytic astrocytoma	hypothalamic tumor
20	2	M	intractable epilepsy	Non-MCD	gliosis	right temporal lobe
21	10	F	tumor	MCD	ganglioglioma	right frontal region

Table A.1 (cont). Clinical characteristics of the intractable epilepsy patients and controls

Sample	Age (years)	Gender	Initial Diagnosis	MCD Status	Pathology	Location in brain
22	6	M	tumor	Non-MCD	pilocytic astrocytoma, glioblastoma, hypothalamic tumor	hypothalamus
23	7	F	intractable epilepsy	MCD	cortical dysplasia with Rasmussen's encephalitis	left anterior temporal lobe, left uncal region, left hippocampus
24	4	M	intractable epilepsy	MCD	cortical dysplasia, type IIA	left frontal temporal lobe
25	4	F	intractable epilepsy	MCD	tuberous sclerosis	left temporal lobe
26	19	F	tumor	Non-MCD	oligodendroglioma	left temporoparietal region
27	16	F	intractable epilepsy	MCD	cortical dysplasia, type IIB	left parietal area
28	2	M	tumor	Non-MCD	medulloblastoma	right cerebellum
29	4	F	tumor	Non-MCD	pilocytic astrocytoma, hypothalamic tumor	hypothalamus
30	5	M	intractable epilepsy	MCD	glioneuronal neoplasm/possible ganglioglioma	right frontal lobe
31	N/A	N/A	intractable epilepsy	Non-MCD	Information not available	Information not available
32	9	F	tumor	Non-MCD	pilocytic astrocytoma	posterior fossa
33	N/A	N/A	intractable epilepsy	Non-MCD	Information not available	Information not available
34	3	M	intractable epilepsy	MCD	cortical dysplasia	parieto-occipital lobe
35	13	M	tumor	Non-MCD	medulloblastoma	posterior fossa
36	16	F	tumor	Non-MCD	ependymoma	left cerebellar medullary
37	2	F	intractable epilepsy	MCD	cortical dysplasia with Rasmussen's encephalitis	right frontotemporal parietal region
38	17	F	intractable epilepsy	Non-MCD	Information not available	left temporal lobe
39	12	F	tumor	Non-MCD	medulloblastoma	IV ventricle

Table A.1 (cont). Clinical characteristics of the intractable epilepsy patients and controls

Sample	Age (years)	Gender	Initial Diagnosis	MCD Status	Pathology	Location in brain
40	10	F	intractable epilepsy	MCD	cortical dysplasia, Palmini type IA	right temporal lobe
41	7	M	tumor	Non-MCD	anaplastic ependymoma	IV ventricle
42	3	F	intractable epilepsy	MCD	cortical dysplasia, type 1A	left frontal lobe, hippocampus
43	9	M	intractable epilepsy	MCD	neuronal disorganization, Type 1a cortical dysplasia	fronto-parietal
44	6	F	tumor	Non-MCD	high grade malignant undifferentiated neoplasm	Information not available
45	N/A	N/A	intractable epilepsy	Non-MCD	Information not available	Information not available
46	4	F	tumor	MCD	DNET	right temporal region
47	4 mos	M	tumor	MCD	cortical dysplasia, type 2B	left temporoparietal cortex
48	6	M	intractable epilepsy	MCD	cortical dysplasia, Palmini type 1B	parietal cortex
Control 1	15	F	Control Sample	Diseased	car accident, multiple injuries	frontal temporal region
Control 2	18	F	Control Sample	Diseased	car accident, multiple injuries	prefrontal cortex
Control 3	13	F	Control Sample	Diseased	asphyxia by hanging	parietal lobe
Control 4	2	F	Control Sample	Diseased	drowning	frontal lobe
Control 5	2	F	Control Sample	Diseased	drowning	parietal lobe
Control 6	8	F	Control Sample	Diseased	asphyxia and multiple injuries	temporal lobe
Control 7	4	F	Control Sample	Diseased	lymphocytic myocarditis	temporal lobe
Control 8	16	F	Control Sample	Diseased	car accident, multiple injuries	parietal lobe
Control 9	2	F	Control Sample	Diseased	car accident, multiple injuries	temporal lobe
Control 10	17	F	Control Sample	Diseased	car accident, multiple injuries	temporal lobe
Control 11	10	F	Control Sample	Diseased	asthma	temporal lobe

Table A.2.
Mitochondrial copy number relative to 18s rRNA gene content as determined from the Ct differences

Sample	18s rRNA Ct	mtDNA Ct	ΔCt[a]	R_c[b]	logR_c
1	21.43	16.60	4.83	28.44	1.45
2	22.51	20.20	2.31	4.96	0.70
3	24.40	19.60	4.80	27.76	1.44
4	27.83	23.63	4.20	18.32	1.26
5	17.25	15.04	2.21	4.63	0.67
6	21.92	16.62	5.30	39.26	1.59
7	16.77	12.00	4.78	27.38	1.44
8	23.47	17.34	6.13	70.03	1.85
9	23.75	17.07	6.68	102.18	2.01
10	25.26	18.97	6.29	77.98	1.89
11	24.76	18.50	6.27	76.90	1.89
12	24.94	20.99	3.95	15.45	1.19
13	24.37	18.82	5.55	46.85	1.67
14	22.76	22.27	0.49	1.40	0.15
15	26.42	24.49	1.93	3.80	0.58
16	20.53	15.74	4.79	27.67	1.44
17	21.70	17.25	4.45	21.86	1.34
18	23.96	22.18	1.78	3.42	0.53
19	24.49	17.37	7.13	139.58	2.14
20	31.35	24.52	6.83	113.38	2.05
21	25.84	18.16	7.68	204.36	2.31
22	19.63	16.84	2.80	6.94	0.84
23	17.23	12.30	4.93	30.38	1.48
24	21.12	15.13	5.99	63.34	1.80
25	33.30	25.60	7.70	207.94	2.32
26	19.37	13.99	5.39	41.79	1.62
27	22.37	15.98	6.39	83.58	1.92
28	22.69	19.15	3.54	11.59	1.06
29	19.74	24.44	-4.70	0.04	-1.41
30	25.21	17.73	7.48	178.53	2.25
31	23.14	13.81	9.33	643.59	2.81
32	17.67	15.21	2.46	5.48	0.74

[a]$\Delta Ct = Ct_{18s\ rRNA} - Ct_{mtDNA\ (ND1)}$

[b]R_c, Relative mtDNA copy number

Table A.2. (cont.)
Mitochondrial copy number relative to 18s rRNA gene content as determined from the Ct differences

Sample	18s rRNA Ct	mtDNA Ct	ΔCt[a]	R_c[b]	logR_c
33	25.89	16.46	9.44	692.18	2.84
34	25.14	18.07	7.07	134.36	2.13
35	20.08	14.35	5.73	53.08	1.72
36	24.15	17.26	6.90	119.02	2.08
37	32.08	24.01	8.08	269.66	2.43
38	24.63	18.52	6.11	69.07	1.84
39	36.57	25.49	11.08	2157.28	3.33
40	30.34	24.22	6.13	69.79	1.84
41	32.39	23.03	9.36	654.84	2.82
42	34.69	23.04	11.65	3202.54	3.51
43	37.00	21.88	15.13	35733.76	4.55
44	32.69	16.88	15.81	57250.47	4.76
45	36.57	22.73	13.84	14613.36	4.16
46	35.92	23.48	12.44	5537.43	3.74
47	25.20	19.58	5.62	49.18	1.69
48	29.13	19.19	9.94	978.89	2.99
Control 1	20.12	15.17	4.95	30.91	1.49
Control 2	19.4	14.52	4.88	29.45	1.47
Control 3	21.75	16.66	5.09	33.94	1.53
Control 4	19.13	15.5	3.63	12.34	1.09
Control 5	20.16	15.24	4.92	30.27	1.48
Control 6	18.91	13.41	5.5	45.25	1.66
Control 7	16.96	13.18	3.78	13.74	1.14
Control 8	19.35	15.02	4.33	20.04	1.30
Control 9	19.61	15.07	4.54	23.26	1.37
Control 10	19.86	16.48	3.38	10.45	1.02
Control 11	21.2	17.53	3.67	12.77	1.11

[a]$\Delta Ct = Ct_{18s\ rRNA} - Ct_{mtDNA\ (ND1)}$

[b]R_c, Relative mtDNA copy number

Table A.3.
Mitochondrial DNA oxidative damage determined from Ct differences

Sample	ND1 Ct (hOGG1 treated)	ND1 Ct (no hOGG1 treatment)	ΔCt[a]	logΔCt
1	17.80	16.60	1.20	0.08
2	25.38	20.20	5.19	0.71
3	25.57	19.60	5.97	0.78
4	24.51	23.63	0.88	-0.06
5	17.53	15.04	2.50	0.40
6	21.98	16.62	5.36	0.73
7	11.54	12.00	-0.46	---
8	23.70	17.34	6.37	0.80
9	32.95	17.07	15.88	1.20
10	30.85	18.97	11.88	1.07
11	32.82	18.50	14.32	1.16
12	34.97	20.99	13.98	1.15
13	28.31	18.82	9.50	0.98
14	26.60	22.27	4.33	0.64
15	28.24	24.49	3.75	0.57
16	27.13	15.74	11.40	1.06
17	19.97	17.25	2.72	0.43
18	36.29	22.18	14.11	1.15
19	22.02	17.37	4.66	0.67
20	24.32	24.52	-0.20	---
21	22.15	18.16	3.99	0.60
22	19.99	16.84	3.16	0.50
23	21.97	12.30	9.67	0.99
24	16.65	15.13	1.52	0.18
25	23.72	25.60	-1.88	---
26	20.51	13.99	6.53	0.81
27	21.08	15.98	5.10	0.71
28	28.01	19.15	8.86	0.95
29	18.17	24.44	-6.28	---
30	22.60	17.73	4.87	0.69
31	20.12	13.81	6.32	0.80
32	16.92	15.21	1.71	0.23

[a]$\Delta Ct = Ct_{\text{mtDNA (ND1; hOGG1 treated)}} - Ct_{\text{mtDNA (ND1, not treated with hOGG1)}}$
[a]ΔCt, mtDNA oxidative damage

Table A.3. (cont.)
Mitochondrial DNA oxidative damage determined from Ct differences

Sample	ND1 Ct (hOGG1 treated)	ND1 Ct (no hOGG1 treatment)	ΔCt[a]	logΔCt
33	22.13	16.46	5.68	0.75
34	26.83	18.07	8.76	0.94
35	16.98	14.35	2.63	0.42
36	23.28	17.26	6.03	0.78
37	24.27	24.01	0.27	-0.58
38	24.34	18.52	5.82	0.76
39	19.90	25.49	-5.60	---
40	25.24	24.22	1.02	0.01
41	16.83	23.03	-6.21	---
42	23.25	23.04	0.20	-0.69
43	17.16	21.88	-4.72	---
44	14.19	16.88	-2.69	---
45	25.74	22.73	3.01	0.48
46	17.94	23.48	-5.55	---
47	18.06	19.58	-1.53	---
48	26.57	19.19	7.38	0.87
Control 1	19.46	15.17	4.29	0.63
Control 2	17.77	14.52	3.25	0.51
Control 3	22.83	16.66	6.17	0.79
Control 4	20.78	15.5	5.28	0.72
Control 5	18.70	15.24	3.46	0.54
Control 6	18.01	13.41	4.6	0.66
Control 7	15.55	13.18	2.38	0.38
Control 8	15.85	15.02	0.83	-0.08
Control 9	19.35	15.07	4.28	0.63
Control 10	25.11	16.48	8.63	0.94
Control 11	24.10	17.53	6.57	0.82

[a]ΔCt= Ct mtDNA $_{(ND1;\ hOGG1\ treated)}$ - Ct $_{mtDNA\ (ND1,\ not\ treated\ with\ hOGG1)}$
[a]ΔCt, mtDNA oxidative damage

Table A.4.
Comparison of Malformation of Cortical Development (MCD) intractable epilepsy patients and control group

	MCD (n=18)	Control (n=11)	*P*-value
Age (years)	7.28 ± 5.47	9.73 ± 6.44	0.28
Sex (M/F)	8/10	0/11	0.01
log Relative mtDNA copy number			
Median	2.03	1.37	
Mean ± S.D.	2.24 ± 0.92	1.33 ± 0.21	0.001
> 1.53 (high) (%)	13 (72)	1 (9)	0.004
≤ 1.53 (low) (%)	5 (28)	9 (82)	
log Degree of oxidative mtDNA damage (ΔCt)			
Median	0.69	0.63	
Mean ± S.D.	0.43 ± 0.60	0.59 ± 0.27	0.39
> 0.65 (high damage) (%)	7 (39)	5 (45)	1.00
≤ 0.65 (low damage) (%)	6 (33)	6 (55)	
log Degree of oxidative mtDNA damage/ log Relative mtDNA copy number			
Median	0.29	0.42	
Mean ± S.D.	0.27 ± 0.34	0.46 ± 0.25	0.13
> 0.37 (high damage) (%)	4 (22)	7 (64)	0.22
≤ 0.37 (low damage) (%)	9 (50)	4 (36)	

Table A.5.
Comparison of Non Malformation of Cortical Development intractable epilepsy patients and control group

	Non-MCD (n=27)	Control (n=11)	*P*-value
Age (years)	8.57 ± 4.86	9.73 ± 6.44	0.56
Sex (M/F)	11/13	0/11	0.02
log Relative mtDNA copy number			
Median	1.67	1.37	
Mean ± S.D.	1.73 ± 1.24	1.33 ± 0.21	0.12
> 1.49 (high) (%)	16 (59)	2 (18)	0.06
≤ 1.49 (low) (%)	11 (41)	8 (73)	
log Degree of oxidative mtDNA damage (ΔCt)			
Median	0.72	0.63	
Mean ± S.D.	0.67 ± 0.26	0.59 ± 0.27	0.47
> 0.67 (high damage) (%)	12 (44)	4 (36)	0.47
≤ 0.67 (low damage) (%)	10 (37)	7 (64)	
log Degree of oxidative mtDNA damage/ log Relative mtDNA copy number			
Median	0.45	0.42	
Mean ± S.D.	0.66 ± 0.86	0.46 ± 0.25	0.48
> 0.44 (high damage) (%)	11 (41)	5 (45)	1.00
≤ 0.44 (low damage) (%)	11 (41)	6 (55)	

Table A.6.
Comparison of Malformation of Cortical Development intractable epilepsy patients and Non-MCD epilepsy patients

	MCD (n=18)	Non-MCD (n=27)	*P*-value
Age (years)	7.28 ± 5.47	8.57 ± 4.86	0.42
Sex (M/F)	8/10	11/13	1.00
log Relative mtDNA copy number			
Median	2.03	1.67	
Mean ± S.D.	2.24 ± 0.92	1.73 ± 1.24	0.15
> 1.84 (high) (%)	10 (56)	10 (37)	0.23
≤ 1.84 (low) (%)	7 (39)	16 (59)	
log Degree of oxidative mtDNA damage (ΔCt)			
Median	0.69	0.72	
Mean ± S.D.	0.43 ± 0.60	0.67 ± 0.26	0.20
> 0.71 (high damage) (%)	8 (44)	12 (44)	0.49
≤ 0.71 (low damage) (%)	5 (28)	10 (37)	
log Degree of oxidative mtDNA damage/ log Relative mtDNA copy number			
Median	0.29	0.45	
Mean ± S.D.	0.27 ± 0.34	0.66 ± 0.86	0.13
> 0.38 (high damage) (%)	4 (22)	13 (48)	0.16
≤ 0.38 (low damage) (%)	9 (50)	9 (33)	

Table A.7.
Mitochondrial SNPs and haplogroups

Region	Variant	Reported in Patients
12S rRNA	G709A	Non-syndromic hearing loss
12S rRNA	T710C	Colorectal tumor, mtDNA haplogroup (Hg)-L1b
12S rRNA	A1555G	Maternally inherited deafness or aminoglycoside-induced deafness
16S rRNA	G1719A	Hg-I, X
16S rRNA	T1738C	colorectal tumor
16S rRNA	G3196A	Alzheimer's Disease and Parkinson's Disease (ADPD)
16S rRNA	T3197C	Hg-U5
ND1	T3308C	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS); colorectal tumor
ND1	T3394C	Leber Hereditary Optic Neuropathy (LHON); Non-insulin dependent diabetes mellitus (NIDDM); acute leukemia
ND1	C3594T	Hg-L (L1 or L2)
ND1	A3397G	ADPD
ND1	A4136G	LHON
tRNA Ile	A4295G	Maternally inherited hypertropic cardiomyopathy (MHCM)
tRNA Ile	G4298A	Chronic Progressive External Ophthalmoplegia (CPEO) / Multiple Sclerosis (MS)
tRNA Ile	G4309A	CPEO
tRNA Ile	A4317G	Fatal Infantile Cardiomyopathy (FICP)
tRNA Gln	T4336C	ADPD
ND2	A4917G	LHON, Hg-T
COX II	G8251A	Sensorineural Hearing Loss (SNHL); Hg-I,W
ATP6	G8994A	SNHL; Hg-W
ATP6	G9055A	Hg-K, longevity, ↓PD
CO III	G9438A	LHON
CO III	G9738T	LHON
CO III	G9804A	LHON
CO III	G9952A	Mitochondrial Encephalopathy
CO III	T9957C	Progressive encephalopathy (PEM); MELAS
tRNA Gly	T9997C	MHCM
tRNA Gly	A10006G	Chronic intestinal pseudo-obstruction (CIPO)
tRNA Gly	T10010C	PEM
tRNA Gly	T10034C	Hg-I
tRNA Gly	A10044G	Gastroesophageal reflux (GER) /Sudden infant death syndrome (SIDS)
ND3	A10398G	↓PD, ↓AD;A-↑Breast Cancer (BRCA) in AA, Hg-I, J, L, M
ND3	C10400T	Hg-M
tRNA LeuCUN	A12308G	Hg-U&K; CPEO / Stroke / Cardiomyopathy (CM)
ND5	G13368A	Hg-T
ND5	G13708A	Hg-J; LHON

Table A.8.
mtDNA haplogroups J, L, M, T, U, and X

Haplogroups	SNPs
L	10398G
J	4216C, 10398G, 13708A, 16069T
L0, L1, L2	3594T
L3	3594C
M	10398G
T	4216C, 7028T, 10398A, 12308G, 13368A, 15607G
U	9055G, 10398A, 12308G
X	1719X, 7028T, 10398A, 14470C

Table A.9.
Sequences for amplifying mitochondrial genome

Name	Forward Primers, Sequence (5' - 3')	Name	Reverse Primers, Sequence (5' - 3')	Amplicon Size (bp)
MtF537	CATACCCCGAACCAACCA	MtR828	GTTAATCACTGCTGTTTCC	292
MtF1351	GCAAGAAATGGGCTACAT	MtR1762	TATCTATTGCGCCAGGTT	412
F1672	CTAAACCTAGCCCCAAACC	R1895	GCTTTGGCTCTCCTTGCAA	224
MtF3116	CCTCCCTGTACGAAAGGAC	MtG3460na	GAGTTTTATGGCGTCAGCGAA	356
F3212	CACCCAAGAACAGGGTTTGT	R3758	AGTAGAATGATGGCTAGGGTGAC	546
MtF4013	CCCTCACCACTACAATCTT	MtR4490	GATGGTAGAGTAGATGACG	478
F4103	CCCTCACCACTACAATCTT	4917na	GCTTACGTTTAGTGAGGGA	825
MtF4881	CCCATCTCAATCATATACC	MtR5501	TAGTATAAAAGGGGAGATAGG	621
MtF5460	GCC CTTACCACGCTA CTCC	MtR5843	TTAGGCCTCTTTTTACCAGC	384
F6872	ACTCGCCACACTCCACGG	R7282	GAATGA GCCTACAGATGA T	409
F8209	CATCGTCCTAGAATTAATTCC	R9169	TGAAAA CGTAGGCTTGGA T	961
MtF9402	ACATACCAAGGCCACCACAC	MtR10108	AGTAGTAAGGCTAGGAGGG	707
G9952ns	GATGTGGTT TGACTATTTC TG	R10629	GCA CAA TAT TGG CTA AGA G	688
F12093	TCCTCCTATCCCTCAACCCC	R12360	GGTTATAGTAGTGTGCATG	268
F14437	AGGATACTCCTCAATAGC C	R15185	GGCGGATAGTAAGTTTGT	766
MtF15539	CCTCCCCACATCAAGCCC	MtR15964	TTTCTCTGATTTGTCCTTGG	426

Table A.10.
Sequences for the mtDNA SNPs and haplogroups

Oligonucleotide Name	Oligo sequence (5' to 3')	Oligonucleotide Name	Oligo sequence (5' to 3')
709-10-ns	GCATCCCCGTTCCAGTGA	T4274C-ms	TAAGAAATATGTCCGATAAAAG
G709A-ms	CATCCCCATTCCAGTGAG	T4285-ns	GATAAAAGAGTTACTTTGATAG
T710C-ms	CATCCCCGCTCCAGTGA	T4285C-ms	GATAAAAGAGCTACTTTGATAG
A1555-ns	TACGACTTGTCTCCTCTAT	A4295-na	CTATTATTTACTCTATCAAAGTA
A1555G-ms	TACGACTTGCCTCCTCTAT	A4295G-ma	CTATTATTTACTCCATCAAAGTA
G1719-ns	ACAACCTTAGCCAAACCAT	G4298A-ma	CTATTATTTATTCTATCAAAGTA
G1719A-ms	ACAACCTTAACCAAACCAT	A4300G-ma	CTATTATTCACTCTATCAAAGTA
A1738-ns	TTTACCCAAATAAAGTATAGG	G4309-na	TAAGGGGGTTTAAGCTCCTAT
T1738C-ms	TTACCCAAACAAAGTATAGG	G4309A-ma	TAAGGGGGTTTAAGCTTCTAT
G3196-ns	CTCAACTTAGTATTATACC	A4317G-ma	TAAGGGGGCTTAAGCTCCTA
G3196A-ms	CTCAACTTAATATTATACC	C4320T-ma	TAAGGAGGTTTAAGCTCCTAT
T3197C-ms	CTCAACTTAGCATTATACCC	T4336-ns	TTCTAGGACTATGAGAATC
T3308-ns	TTAACAACATACCCATGGC	T4336C-ms	TTCTAGGACCATGAGAATC
T3308C-ms	TAACAACACACCCATGGC	T4409-ns	CTAAAGTAAGGTCAGCTAAATA
T3394--ns	TAGGCTATATACAACTAACG	T4409C-ms	CTAAAGTAAGGCCAGCTAAAT
T3394C-ms	ATTCTAGGCCATATACAACT	G4450-ns	GAAAATGTTGGTTATACCCTT
C3594-ns	ACCCCCTGGTCAACCTCA	G4450A-ms	GAAAATGTTGATTATACCCTT
C3594T-ms	ACCCCCTGGTTAACCTCA	A4529-ns	CTCATCACAGCGCTAAGC
A3397-na	GTAGTTGTATATAGCCTAG	A4529T-ms	CTCATCACTGCGCTAAGC
A3397G-ma	GTAGTTGTACATAGCCTAG	G4580-na	TAAAAGCTAGCATGTTTATTTC
A4136-na	ATCGGGGGTATGCTGTTC	G4580A-ma	TAAAAGCTAGTATGTTTATTTC
A4136G-ma	ATCGGGGGCATGCTGTTC	A4917-na	GCTTACGTTTAGTGAGGGA
T4160-ns	ACGACCAACTCATACACCT	A4917G-ma	GCTTACGTCTAGTGAGGGA
T4160C-ms	ACGACCAACCCATACACCT	G5460-ns	ACACTCATCGCCCTTACCA
T4216-ns	TACTTATATGATATGTCTCCAT	G5460A-ms	CACTCATCACCCTTACCAC
T4216C-ms	TACTTATATGACATGTCTCCAT	G5460T-ms	CACTCATCTCCCTTACCAC
A4269-ns	TAAGAAATATGTCTGATAAAAG	G5521-ns	ATAGAAATTTAGGTTAAATACAG
A4269G-ms	TAAGAAATGTGTCTGATAAAAG	G5521A-ms	ATAGAAATTTAAGTTAAATACAG

Table A.10. (cont.)
Sequences for the mtDNA SNPs and haplogroups

Oligonucleotide Name	Oligo sequence (5' to 3')	Oligonucleotide Name	Oligo sequence (5' to 3')
A5537-ns	ATACAGACCAAGAGCCTTCA	G9952-ns	GATGTGGTTTGACTATTTCTG
A5537insT-ms	ATACAGACCATAGAGCCTTC	G9952A-ms	GATGTGGTTTAACTATTTCTG
G5549-ns	AGCCTTCAAAGCCCTCAG	T9957C-ms	ATGTGGTTTGACTACTTCTG
G5549A-ms	AGCCTTCAAAACCCTCAG	T9997-ns	GTCTTACTCTTTTAGTATAAATA
T5692-ns	CAAACACTTAGTTAACAGCT	T9997C-ms	GTCTTACTCTTCTAGTATAAAT
T5692C-ms	CAAACACTTAGCTAACAGCT	A10006-na	AGTTAACGGTACTATTTATACT
T5814-ns	TATGAAAATCACCTCGGAG	A10006G-ma	GTTAACGGTACTACTTATACT
T5814C-ms	TATGAAAACCACCTCGGAG	T10010C-ma	GTTAACGGTGCTATTTATACT
C7028-ns	CGTTGTAGCCCACTTCCA	T10034-ns	TTCCAATTAACTAGTTTTGAC
C7028T-ms	CGTTGTAGCTCACTTCCA	T10034C-ms	TTCCAATTAACTAGCTTTGAC
G8251-na	AAATACGGGCCCTATTTCAA	A10044-na	CTCTTTTTTGAATGTTGTCAAA
G8251A-ma	AAATACGGGTCCTATTTCAA	A10044G-ma	CTCTTTTTTGAACGTTGTCAA
G8994-na	CGTACGGCCAGGGCTATT	A10398-na	TACCAATTCGGTTCAGTCT
G8994A-ma	CGTACGGCTAGGGCTATT	A10398G-ma	TACCAATTCGGCTCAGTCT
G9055-na	CTAGGGTGGCGCTTCCA	C10400T-ma	TACCAATTCAGTTCAGTCT
G9055A-ma	CTAGGGTGGTGCTTCCA	A12308-na	CCAAAATTTTTGGGGCCTA
G9438-ns	GTCCAAAAAGGCCTTCGATA	A12308G-ma	CCAAAATTCTTGGGGCCTA
G9438A-ms	GTCCAAAAAAGCCTTCGATA	G13368-na	ATGATGGACCCGGAGCAC
G9738-ns	CTCCTACAAGCCTCAGAGT	G13368A-ma	ATGATGGATCCGGAGCAC
G9738T-ms	CTCCTACAATCCTCAGAGT	G13708-na	TTCCGGCTGCCAGGCGTT
G9804-ns	TTTTGTAGCCACAGGCTTC	G13708A-ma	TTCCGGCTGTCAGGCGTT
G9804A-ms	TTTTTGTAACCACAGGCTTC		

Table A.11.
Hybridization temperatures for ASO Dot Blots

Probe	Tm (C°)	Probe	Tm (C°)	Probe	Tm (C°)
709-10-ns	47	T4336-ns	52	G9952-ns	58
G709A-ms	47	T4336C-ms	54	G9952A-ms	52
T710C-ms	47	T4409-ns	54	T9957C-ms	56
A1555-ns	46	T4409C-ms	58	T9997-ns	56
A1555G-ms	46	G4450-ns	56	T9997C-ms	56
G1719-ns	47	G4450A-ms	54	A10006-na	56
G1719A-ms	47	A4529-ns	56	A10006G-ma	56
A1738-ns	47	A4529T-ms	56	T10010C-ma	56
T1738C-ms	47	G4580-na	56	T10034-ns	54
G3196-ns	46	G4580A-ma	54	T10034C-ms	56
G3196A-ms	47	A4917-na	56	A10044-na	56
T3197C-ms	46	A4917G-ma	50	A10044G-ma	56
T3308-ns	47	G5460-ns	58	A10398-na	54
T3308C-ms	47	G5460A-ms	58	A10398G-ma	54
T3394--ns	47	G5460T-ms	58	C10400T-ma	52
T3394C-ms	47	G5521-ns	56	A12308-na	56
C3594-ns	47	G5521A-ms	54	A12308G-ma	56
C3594T-ms	47	A5537-ns	58	G13368-na	58
A3397-na	47	A5537insT-ms	58	G13368A-ma	56
A3397G-ma	47	G5549-ns	56	G13708-na	60
A4136-na	56	G5549A-ms	54	G13708A-ma	58
A4136G-ma	58	T5692-ns	40		
T4160-ns	56	T5692C-ms	54		
T4160C-ms	58	T5814-ns	54		
T4216-ns	56	T5814C-ms	56		
T4216C-ms	58	C7028-ns	56		
A4269-ns	52	C7028T-ms	52		
A4269G-ms	54	G8251-na	56		
T4274C-ms	64	G8251A-ma	54		
T4285-ns	56	G8994-na	58		
T4285C-ms	58	G8994A-ma	56		
A4295-na	56	G9055-na	67		
A4295G-ma	58	G9055A-ma	54		
G4298A-ma	54	G9438-ns	64		
A4300G-ma	58	G9438A-ms	56		
G4309-na	60	G9738-ns	54		
G4309A-ma	58	G9738T-ms	56		
A4317G-ma	60	G9804-ns	56		
C4320T-ma	58	G9804A-ms	56		

Table A.12.
The results of the Fisher exact test for individual SNP for Malformations of Cortical Development (MCD) and Non-MCD pediatric intractable epilepsy patients

SNPs	MCD (n=19)		Non-MCD (n=29)		Crude OR	95% CI		Fisher's exact test *P* value
	Positive	%	Positive	%		Lower	Upper	
G709A	18	95	29	100	0	0	NaN	0.40
T710C	19	100	29	100	0	0	NaN	1.00
A1555G	12	63	17	59	1.21	0.37	3.98	0.77
G1719A	1	5	1	3	1.56	0.09	26.47	1.00
T1738C	3	16	3	10	1.63	0.29	9.05	0.67
G3196A	6	32	4	14	2.88	0.69	12.07	0.16
T3197C	0	0	0	0	NaN	NaN	NaN	1.00
T3308C	1	5	0	0	0	0	NaN	0.40
T3394C	29	100	19	100	NaN	NaN	NaN	1.00
C3594T	1	5	0	0	0	0	NaN	0.40
A3397G	1	5	0	0	0	0	NaN	0.40
A4136G	19	100	29	100	NaN	NaN	NaN	1.00
A4317G	13	68	24	82	0.45	0.12	1.77	0.30
A4295G	0	0	1	3	0	0	NaN	1.00
G4309A	16	84	28	97	0.19	0.02	1.99	0.29
T4336C	0	0	0	0	NaN	NaN	NaN	1.00
A4917G	18	95	27	93	1.33	0.11	15.82	1.00
G8251A	0	0	1	3	0	0	NaN	1.00
G8994A	0	0	2	7	0	0	NaN	1.00

NaN = Not a number

Table A.12. (cont.).
The results of the Fisher exact test for individual SNP for Malformations of Cortical Development (MCD) and Non-MCD pediatric intractable epilepsy patients

SNPs	MCD (n=19)		Non-MCD (n=29)		OR	95% CI		Fisher's exact test *P* value
	Positive/ haplogroup	%	Positive/ haplogroup	%		Lower	Upper	
G9438A	1	5	1	3	1.56	0.09	26.47	1.00
G9738T	0	0	2	7	0	0	NaN	0.51
G9804A	1	5	1	3	1.47	0.09	25.03	1.00
G9952A	5	26	13	45	0.32	0.13	1.54	0.24
T9957C	10	53	19	66	0.58	0.18	1.91	0.55
T9997C	11	58	19	66	0.72	0.22	2.38	0.76
A10006G	2	11	7	24	0.37	0.07	2.01	0.29
T10010C	8	42	16	55	0.59	0.18	1.90	0.56
T10034C	16	84	26	90	0.62	0.11	3.43	0.67
A10044G	8	42	14	48	0.78	0.24	2.50	0.77
A10398G	3	16	5	17	0.90	0.19	4.30	1.00
C10400T	5	26	7	24	1.12	0.30	4.24	0.56
A12308G	18	95	27	93	1.33	0.11	15.82	1.00
G13368A	19	100	29	100	NaN	NaN	NaN	1.00
G13708A	14	74	22	76	0.89	0.24	3.37	1.00

NaN = Not a number

Table A.13.
The results of the Fisher exact test for individual haplogroups for Malformations of Cortical Development (MCD) and Non-MCD pediatric intractable epilepsy patients

Haplogroups	MCD (n=19)		Non-MCD (n=29)		OR	95% CI		Fisher's exact test *P* value
	Positive/ haplogroup	%	Positive/ haplogroup	%		Lower	Upper	
I	14	74	22	76	0.93	0.24	3.37	1.00
J	14	74	22	76	0.93	0.24	3.37	1.00
L	1	5	0	0	NaN	NaN	NaN	0.40
M	5	26	7	24	1.12	0.30	4.24	1.00
X	1	5	1	3	1.56	0.09	26.47	1.00
T	18	95	27	93	1.33	0.11	15.82	1.00
U	18	95	27	93	1.33	0.11	15.82	1.00

NaN = Not a number

Table A.14.
Characteristics of Miami Children's Hospital neoplastic intractable epilepsy patients

Sample	Pathology	WHO Classification	Tumor Classification	Tumor Grade
1	new tumor dense gliosis	I	N/A	Low
2	pilocytic astrocytoma	I	astrocytoma	Low
3	ganglioglioma	I/II	astrocytoma	Low
4	ganglioglioma	I/II	astrocytoma	Low
5	oligodendroglioma	II	oligodendroglioma	Low
6	pilocytic astrocytoma	I	astrocytoma	Low
8	pilocytic astrocytoma	I	astrocytoma	Low
12	Dysembryoplastic Neuroepithelial Tumor (DNET)	I	DNET	Low
13	glioblastoma multiforme	IV	astrocytoma	High
14	medulloblastoma	IV	medulloblastoma	High
15	pilocytic astrocytoma	I	astrocytoma	Low
16	astrocytoma	I	astrocytoma	Low
17	choroid plexus papilloma	I	glioma	Low
19	pilocytic astrocytoma	I	astrocytoma	Low
21	ganglioglioma	I/II	astrocytoma	Low
22	pilocytic astrocytoma, glioblastoma, hypothalamic tumor	IV	astrocytoma	High
26	oligodendroglioma	II	oligodendroglioma	Low
28	medulloblastoma	IV	medulloblastoma	High
29	pilocytic astrocytoma, hypothalamic tumor	I	astrocytoma	Low
30	glioneuronal neoplasm/possible ganglioglioma	I/II	astrocytoma	Low
32	pilocytic astrocytoma	I	astrocytoma	Low
35	medulloblastoma	IV	medulloblastoma	High
36	ependymoma	II	ependymoma	Low
39	medulloblastoma	IV	medulloblastoma	High
41	anaplastic ependymoma	III	ependymoma	Low
44	high grade malignant undifferentiated neoplasm	IV	N/A	High
46	DNET	I	DNET	Low

Table A.15.
Comparison of demographic, and mitochondrial DNA copy number and oxidative damage in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients by tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

	All Cases (n=38)	Tumor Low Grade (n=20)	Tumor High Grade (n=7)	Control (n=11)	[a]*P*-value	[b]*P*-value	[c]*P*-value
Age (years)	8.93 ± 5.36	8.57 ± 5.31	8.71 ± 4.11	9.73 ± 6.44	0.95	0.59	0.72
Sex (M/F)	12/26	9/11	3/4	0/11	1.0	0.01	0.04
log Relative mtDNA copy number							
Median	1.45	1.45	1.67	1.37			
Mean ± S.D.	1.53 ± 1.00	1.49 ± 1.03	1.93 ± 1.59	1.33 ± 0.21	0.40	0.62	0.23
> 1.45 (high) (%)		9 (45)	4 (57)	5 (45)	1.00	1.00	1.00
≤ 1.45 (low) (%)		10 (20)	3 (43)	6 (55)			
log Degree of oxidative mtDNA damage (ΔCt)							
Median	0.66	0.69	0.64	0.63			
Mean ± S.D.	0.62 ± 0.29	0.61 ± 0.32	0.69 ± 0.26	0.59 ± 0.27	0.60	0.87	0.49
> 0.66 (high damage) (%)		9 (45)	2 (29)	5 (45)	0.64	0.69	0.71
≤ 0.66 (low damage) (%)		7 (35)	3 (43)	6 (55)			
Degree of oxidative mtDNA damage/ log Relative mtDNA copy number							
Median	0.46	0.44	0.59	0.42			
Mean ± S.D.	0.60 ± 0.73	0.48 ± 0.31	1.33 ± 1.70	0.46 ± 0.25	0.05	0.89	0.32
> 0.46 (high damage) (%)		9 (45)	2 (29)	6 (55)	0.64	0.69	0.28
≤ 0.46 (low damage) (%)		7 (35)	3 (43)	4 (36)			

[a]**P-value for Brain Tumor Low Grade (WHO Tumor Grading ≤ 2) vs. High Grade (WHO Tumor Grading > 2)**
[b]**P-value for Brain Tumor Low Grade vs. Control**
[c]**P-value for Brain Tumor High Grade vs. Control**

Table A.16.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with high tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	High Tumor Grade (n=7)		Control (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	3	43	11	100	0.04	0	0, NaN	0	0, NaN
G3196A	2	29	11	100	0.002	0	0, NaN	0	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	0	0, NaN
G4309A	7	100	11	100	1.00	1.62E9	0, NaN	1.46E9	0, NaN
A4317G	7	100	11	100	1.00	1.62E9	0, NaN	1.46E9	0, NaN
G9804A	0	0	0	0	1.00	NaN	0, NaN	NaN	NaN, NaN
G9952A	1	14	0	0	0.39	0	0, NaN	0	0, NaN
A10006G	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
T10010C	3	43	7	64	0.63	0.42	0.06, 2.97	0.44	0.32, 15.93
A10398G	0	0	11	100	<0.001	0	0, NaN	0	0, NaN

[a]OR, Crude Odds ratio using control as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table A.17.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients by high tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	High Tumor Grade (n=7)		Control (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-J	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-L	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-M	0	0	11	100	<0.001	0	0, NaN	0	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	0	0, NaN

[a]OR, Crude Odds ratio using control as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table A.18.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients by high tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

SNP/Haplogroup	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	7.12E9 (0, NaN)	0.99 (0.04, 23.15)	1.42 (0.08, 25.70)	0.92 (0.73, 1.17)
G3196A	74.91E25 (0, NaN)	3.16E8 (0, NaN)	2.60E8 (0, NaN)	1.01 (0.72, 1.66)
T3197C	3.61E17 (0, NaN)	0 (0, NaN)	1.42 (0.03, 77.68)	0.90 (0.63, 1.28)
G4309A	---	0.81 (0.07, 9.09)	0.99 (0.11, 9.07)	0.96 (0.78, 1.17)
A4317G	---	0.81 (0.07, 9.09)	0.99 (0.11, 9.07)	0.96 (0.78, 1.17)
G9804A	---	0.81 (0.07, 9.09)	0.99 (0.11, 9.07)	0.96 (0.78, 1.17)
G9952A	0 (0, NaN)	0.40 (0.03, 6.30)	0.57 (0.05, 6.51)	0.92 (0.73, 1.16)
A10006G	---	---	---	---
T10010C	0.80 (0.06, 10.77)	0.72 (0.04, 11.79)	0.99 (0.11, 9.12)	0.95 (0.76, 1.18)
A10398G	---	---	---	---
Hg-I	---	---	---	---
Hg-J	---	---	---	---
Hg-L	---	---	---	---
Hg-M	---	---	---	---
Hg-U5	3.61E17 (0, NaN)	0 (0, NaN)	1.42 (0.03, 77.68)	0.90 (0.63, 1.28)

OR, using control group as reference
NaN = Not a number

Table A.19.
The results of the Fisher's exact test for individual SNP loci for in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients with low tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

SNPs	Low Tumor Grade (n=20)		Control (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ SNP	%	Positive/ SNP	%					
A1555G	9	45	11	100	0.004	0	0, NaN	2.16E9	0, NaN
G3196A	1	5	11	100	<0.001	0	0, NaN	5.25E10	0, NaN
T3197C	0	0	9	82	0.002	0	0, NaN	1.62E10	0, NaN
G4309A	19	95	11	100	1.00	0	0, NaN	8.69E8	0, NaN
A4317G	12	60	11	100	0.03	0	0, NaN	1.44E9	0, NaN
G9804A	0	0	0	0	1.00	NaN	0, NaN	0.96	0.84, 1.11
G9952A	11	55	0	0	0.002	0	0, NaN	0	0, NaN
A10006G	6	30	11	100	<0.001	0	0, NaN	3.29E9	0, NaN
T10010C	13	65	7	64	1.00	1.06	0.23, 4.92	0.94	0.20, 4.37
A10398G	7	35	11	100	<0.001	0	0, NaN	2.23E9	0, NaN

[a]OR, Crude Odds ratio using control as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table A.20.
The results of the Fisher's exact test for haplogroups for in Miami Children's Hospital pediatric intractable epilepsy patients by low tumor grade and non-epileptic control from NICHD Brain and Tissue Bank

Haplogroups	Low Tumor Grade (n=7)		Control (n=11)		Fisher's exact test *P* value (2-sided)	OR[a]	95% Confidence Interval	Adjusted OR[b]	95% Confidence Interval
	Positive/ Hg	%	Positive/ Hg	%					
Hg-I	7	35	11	100	<0.001	0	0, NaN	2.23E9	0, NaN
Hg-J	7	35	11	100	<0.001	0	0, NaN	0, NaN	0, NaN
Hg-L	7	35	11	100	<0.001	0	0, NaN	2.23E9	0, NaN
Hg-M	7	35	11	100	<0.001	0	0, NaN	2.23E9	0, NaN
Hg-U5	0	0	9	82	0.002	0	0, NaN	1.62E10	0, NaN

[a]OR, Crude Odds ratio using control as reference category
[b]ORs adjusted for age using logistic regression
NaN = Not a number

Table A.21.
The results of the logistic regression for SNPs/Hg, level of relative mtDNA copy number, level of oxidative mtDNA damage, and age in Miami Children's Hospital pediatric neoplastic intractable epilepsy patients by low tumor grade using non-epileptic control from NICHD Brain and Tissue Bank as reference

SNP/Haplogroup	SNP/Hg OR (95% CI)	Level of relative mtDNA copy number OR (95% CI)	Level of oxidative mtDNA damage OR (95% CI)	Age OR (95% CI)
A1555G	7.00E9 (0, NaN)	0.34 (0.02, 4.77)	0.16 (0.01, 2.14)	0.95 (0.78, 1.16)
G3196A	---	---	---	---
T3197C	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)
G4309A	---	1.05 (0.18, 6.13)	0.42 (0.08, 2.29)	0.98 (0.84, 1.15)
A4317G	1.69E9 (0, NaN)	0.86 (0.13, 5.92)	0.54 (0.09, 3.42)	1.00 (0.86, 1.17)
G9804A	---	1.05 (0.18, 6.13)	0.42 (0.08, 2.29)	0.98 (0.84, 1.15)
G9952A	0 (0, NaN)	0.17 (0.01, 2.43)	0.45 (0.04, 4.94)	1.00 (0.82, 1.23)
A10006G	1.08E18 (0, NaN)	5.89E8 (0, NaN)	4.06 (0.17, 99.64)	1.05 (0.83, 1.34)
T10010C	0.47 (0.06, 3.70)	0.71 (0.09, 5.84)	0.44 (0.08, 2.43)	0.98 (0.84, 1.14)
A10398G	3.19E9 (0, NaN)	1.15 (0.12, 11.40)	0.27 (0.03, 2.76)	0.95 (0.76, 1.17)
Hg-I	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)
Hg-J	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)
Hg-L	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)
Hg-M	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)
Hg-U5	5.72E17 (0, NaN)	0 (0, NaN)	1.03 (0.03, 39.97)	0.97 (0.71, 1.33)

OR, using control group as reference
NaN = Not a number

DNA ISOLATION PROTOCOL

1. Tissue
    a. Homogenize cells in 1mL TRizol Reagent (under Fume Hood).
    b. Pipette into epi tube (Lyse cells by repetitive pipetting).
2. Phase Separation
    a. Incubate cells/TRizol at 15-30 C for 5 min.
    b. Add 200uL of chloroform (per 1mL TRizol used- under the Fume Hood).
    c. Cap tubes and vortex for 15 seconds.
    d. Incubate at 15-30 C (room temp) for 15 minutes.
    e. Centrifuge samples @ 12K rpm @4 C for 15 min.
    *After centrifugation : aqueous = RNA
    interphase = protein/DNA
    organic = DNA
3. DNA Precipitation
    a. Remove aqueous phase (RNA) into new epi. Tube.
    b. Add 300uL 100% Ethanol (200 proof EtOH) per 1mL TRizol used to interphase/organic phase.
    c. Vortex gently.
    d. Incubate samples at 15-30 C for 2-3 min.
    e. Centrifuge at 12K rpm @ room T for 5 min.
4. DNA Wash
    a. Remove phenol/ethanol sup (contains protein) to waste.
    b. Wash DNA pellet <u>2 times</u> with 0.1M sodium citrate (Add 1mL Na Citrate for every 1mL TRizol used).
    c. Gently vortex.
    d. Incubate samples at 15-30 C for 15 min.
    e. Spin at 12K rpm at room 4C for 5 min.
    f. Resuspend DNA in 1mL 75% Ethanol (per 1mL TRizol used).
    g. Incubate at room T for 10-20 min.
    h. Centrifuge at 12K rpm @ room T for 5 min.
    i. Remover supernatant with p200 pipettor.
    j. Briefly dry pellet for 2-5 min under vacuum (can leave on bench).
5. Redissolving of DNA
    a. Dissolve pellet in 60uL of 1X TE Buffer with a pipette.
    b. Aliquot DNA into three 10uL samples.
    c. Store -80 C.
    d. Run 2% agarose gel (quality)
    e. Check DNA conc. by spec. (quantity)

DOT BLOT PROTOCOL

1. Cut membrane to appropriate size, make a grid with pencil.
2. Denature PCR products by heating at 95°C for 10 minutes, then place on ice.
3. Place 2uL of PCR product on membrane.
4. Let air membrane air dry (~1 hour).
5. UV crosslink membrane for 4 minutes.
6. Dip membrane in ddH2O.
7. Place membrane in pre-heated hybridization solution for 15 minutes.
8. Add DIG-labeled Oligonucleotide (30uL of Miracle Hyb. And 3uL of probe).
9. Hybridize at proper temperature, over night.
10. Place membrane in 2X SSC + 0.1% SDS for 15 minutes, 2 times.
11. Place membrane in 0.1X SSC + 0.1% SDS at hybridization temperature for 30 minutes.
12. Place membrane in washing buffer for 5 minutes.
13. Place membrane in blocking buffer for 30 minutes.
14. Place membrane in anti-DIG solution for 30 minutes.
15. Place membrane in washing buffer for 15 minutes, 2 times.
16. Place membrane in detection solution for 3 minutes.
17. Add 250uL of CSPD to membrane.
18. Place in clear wrap.
19. Place in 37°C for 10 minutes.
20. Visualize in X-Ray developer or VersaDoc® Imager.

VITA

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EDUCATION

1996-2000	Bachelor of Science in Biology and Second Major in Chemistry/ Honors College Program Florida International University Miami, Florida
2002- 2004	Agency Manager, Estrella Insurance
2004- 2006	Masters of Public Health Graduate Certificate in Environmental and Occupational Health Florida International University Miami, Florida
2004-2009	Graduate/Research Assistant, Florida International University's Robert Stempel College of Public Health and Social Work (CPHSW)
2006	Research Assistant, Florida International University's Center on Aging
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PUBLICATIONS AND PRESENTATIONS

McCoy, H.V., Malow, R., Luna, B., Thurland, A., and Edwards, R.W. (2005). Community Readiness Model: Bridging micro and macro-histories to reduce HIV/AIDS stigma. 133rd Annual Meeting American Public Health Association, December 10-14, Philadelphia, PA.

Luna, B. (2006). ABCD Dental Program. *International Pediatrics, 21(1),* 51.
Luna, B. (2006). Metallic Charm and Child Lead Poisoning. *International Pediatrics*, *21(2),* 112.
Luna, B. (2006). Sunscreen Use Low Among U.S. High School Students. *International Pediatrics, 21(3),* 183.
Luna, B. (2006). Maternal Smoking During Pregnancy and Sudden Infant Death. *International Pediatrics, 21(4),* 237.
Seff, L., Rothman, M., Pekovic, V., Davalos, D., and Luna, B. (2007). 2006-2007 Updated Elder Analysis for Broward County: Final Report, Recommendations, and Business Plan. Broward County Department of Human Services Division of Elderly and Veterans Services. April 9, 2007.
Seff, L., Rothman, M., Pekovic, V., Davalos, D., and Luna, B. (2007). Designing A Model All-Hazards Plan For Older Adults: The Role of the Aging Services Network in Assuring Community All-Hazards Readiness for Elders and in Providing Assistance to Elders When Disasters Occur. The University of South Florida, Florida Policy Exchange Center on Aging and the U.S. Administration on Aging. April 20, 2007.
Luna, B. (2007). Siblings of Children with Epilepsy Affected by Disease. *International Pediatrics, 22 (1),* from http://int-pediatrics.org.
Luna, B. (2007). Autism Spectrum Disorders May Increase Risk of Infections in the First Two Years of Life. *International Pediatrics*, 22(1), from http://int_pediatrics.org.
Luna, B. (2007). ADHD and Childhood Epilepsy. *International Pediatrics, 22(2),* From http://intpediatrics.org.
Luna, B., Roy, D., and Felty, Q. (2008). NRF-1 Expression from Different Breast Tissue at Different Stages of Breast Cancer. 1st Annual FIU/UM Braman Family Breast Cancer Institute Breast Cancer Symposium, September 2, Miami, FL.
Luna, B., Bhatia, S., Ragheb, Miller, I., Jayakar, P., et al. (2008). Gene-environment interactions, malformations of cortical development and epilepsy in children. *Encyclopedia of Environmental Health, 73,* IN PRINT.
Luna, B. (2008). Early onset of non-medical use of prescription drugs predicts subsequent prescription drug abuse and dependence. *International Pediatrics*, *23(4),* from http://int-pediatrics.org.
Luna, B. (2009). Increased Energy Expenditure with the use of Activity-Promoting Video Games. *International Pediatrics, 24(3),* from http://intpediatrics.org.
Deoraj, A., Okoh, V., Kunkle, B., Luna, B., Parkash, J., and Roy, D. (2009). Redox Sensitive Gene as a Biomarker for Breast Cancer. 2nd Annual Florida International University Breast Cancer Symposium, June 25, 2009, Miami, FL.
Luna, B., Bhatia, S., Ragheb, J., Jayakar,P., Felty, Q., and Roy, D. (2009). Role Of NRF-1 Regulable Genes In Cortical Lesions In Children. Society of Toxicology's PPTOXII: Role of Environmental Stressors in the Developmental Origins of Disease, December 7-10, 2009, Miami, FL.
Deoraj, A., Okoh, V., Luna, B., Parkash, J., Takata, S., and Roy, D. (2010). NRF-1 Over expression and Growth of Breast Lesions. SOT 49th Annual Meeting, March 7-11, Salt Lake City, UT.