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**Proposal Cover Sheet**

**Term: Fall Year 2011**

**Instructor Dr. Nora E. Demers**

Name: Francesca Montellanos

Present Year in Education (e.g., freshman, sophomore, etc.): Senior

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Major Biology B.S

Have you identified a research mentor for a senior thesis (if applicable)?

\_\_\_\_\_ Yes x No.

If yes, please identify.

Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Title of Proposal:**

“The Relationship between Mitochondrial abnormalities and Hypotonia in Autistic Children: Hypotonia as a comorbid condition due to mitochondrial abnormalities”

Keywords (3-5): Hypotonia, Mitochondrial dysfunction, Autism

**Checklist:**

All required portions of the first submission are included \_x\_\_ Yes \_\_\_\_\_ No

I had an external reviewer read the proposal \_\_\_\_\_ Yes \_\_x\_ No

If Yes, who \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ When \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

I authorize the use of this proposal as an example in future courses \_\_x\_ Yes \_\_\_\_\_ No

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“The Relationship between Mitochondrial abnormalities and Hypotonia in Autistic Children: Hypotonia as a comorbid condition due to mitochondrial abnormalities”

**Abstract:**

Autism is a behavioral phenotype signifying brain dysfunction. In some cases, autism manifests along with different motor impairments that can vary among affected individuals. This study aims to analyze whether Hypotonia, a motor impairment, manifests as a comorbid condition in Autism as a result of mitochondrial abnormalities. Biopsies of skeletal muscle from participants will be homogenized and analyzed for any mitochondrial abnormalities under light microscope. Results will be recorded and compared. The study will involve 15 children (5-12) (participants) who exhibit Autism and different motor impairments, including hypotonia.

**Introduction**

Autism and related autism spectrum disorders (ASDs) are denominated as impairments in social interaction, language and speech development, and the appearance of repetitive behaviors with restricted interests (Ming, Brimacombe, Wagner, 2007). An estimated 1 out of 110 individuals in the United States is currently affected with ASD (Rosignol and Bradstreet, 2011). Autism is a behavioral phenotype signifying brain dysfunction, however it is etiologically heterogeneous. Despite intense research, specific etiology of ASDs remains largely unknown but is likely multifactorial, including biologic, genetic, and environmental factors (Zecavati and Spence, 2009). In some cases, autism manifests along with different motor impairments categorized as ‘‘associated symptoms,’’ including hypotonia, limb apraxia, and toe-walking, among others. Hypotonia is a condition of decreased muscle tone (the amount of resistance to movement in a muscle). Little is known about the etiology, but investigations state that it may be caused by trauma, environmental factors, or by genetic, muscle, or central nervous system disorders, such as Down syndrome, muscular dystrophy, cerebral palsy, Prader-Willi syndrome, myotonic dystrophy (Prasad and Prasad, 2011).

A broadening spectrum of neurologic disorders associated with mitochondrial dysfunctions, including abnormalities in specific enzyme activities, morphology, and DNA integrity, are being reported frequently (Filiano et al, 2002). See figure 1 for an overview of normal mitochondria function. Evidence suggests that mitochondrial dysfunctions may be present in children with motor impairments. The aim of this research is to analyze the relationship between mitochondrial abnormalities and hypotonia in autistic children, in order to determine whether it is a comorbid condition as a result of such dysfuntions.

Figure 1. Mitochondrial function and the electron transport chain (ETC). Abbreviations: ADP, adenosine diphosphate; AKA, a-ketoglutarate; ATP, adenosine triphosphate; CoQ, co-enzyme Q; Cyt C, cytochrome c; e, electron; FAD, flavin adenine dinucleotide; FADH2, reduced FAD; H, hydrogen; LCFA, long chain fatty acid; MCFA, medium chain fatty acid; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; OXPHOS, oxidative phosphorylation; PDC, pyruvate dehydrogenase complex; SCFA, short chain fatty acid; TCA, tricarboxylic acid; I, complex I; II, complex II; III, complex III; IV, complex IV; V, complex V.

**Research Objectives:**

The objective in this research is to analyze the relationship between mitochondrial (Mt) abnormalities and hypotonia in children (5-12) who exhibit an autistic phenotype. The aim is to analyze skeletal muscle biopsy samples from participants and look for any (Mt) abnormality including morphology, enzymatic activity, and DNA. Abnormal findings will help to determine whether or not hypotonia, a motor impairment, is a comorbid condition in individuals with Autism.

**Methods:**

*Study Design:*

The role of mitochondrial disorders in autism has generated much discussion (Zecavati & Spence, 2009). This research will aim to analyze the relationship between mitochondrial abnormalities and a motor impairment condition known as hypotonia in children (5-12) showing an autistic phenotype. Since this study will involve the participation of children, authorization from their parents and/or legal guardians will be required prior to start as well as approval from the Institutional Review Board (IRB) at Florida Gulf Coast University.

All the recruited participants will be children exhibiting an autistic phenotype with ages ranging from 5 to 12 years; evaluation by a pediatric neurologist will be required for a proper diagnosis of Autism based on DSM IV (Diagnostic and Statistical Manual of Mental Disorders) criteria (Ming, Brimacombe & Wagner, 2007). There will be 20 individuals, 5 females and 15 males if possible. Participation of a higher number of male participants will be convenient for this research, since studies have revealed a 4.5: 1 male to female ratio in many cohorts of Autism affected individuals (Rossignol and Frye, 2011).

 Motor impairments that will be included in this study are: hypotonia, motor apraxia, toe-walking, and reduced ankle mobility. These physical findings in the children will be documented by physical examination performed by the pediatric neurologist (Ming et al, 2007); it will be useful to have the parents accompanying the children at all times in order to provide other information regarding the children’s health and medical history. For the purpose of this study “hypotonia” will be defined as “reduced resistance during passive movement in the limbs, manifested as increased joint mobility to passive stretch in both distal (e.g., fingers) and proximal (e.g., sitting in the shape of W)”. “Apraxia” will be defined as impairment of the ability to execute skilled movements and gestures, despite having the desire and the physical ability to perform them. “Toe-walking” will be determined by historical report from the parents, therapists’ records, other physician’s records, or by physical examination performed by the pediatric neurologist during office visits. “Reduced ankle mobility” will be defined as reduced degree of ankle dorsi-flexion with passive stretching of the muscle without producing pain (Ming et al, 2007).

*Data Collection:*

In order to collect data for this research, samples of skeletal muscle will be taken from the 20 individuals following procedures applied by (Filiano et al, 2002). Participants will be recruited through advertising at several medical institutions upon voluntary desire of the parent and/or legal guardian. The samples will be submitted to biochemical analysis. The tissue samples will be first homogenized and then ultrastructural examination of skeletal muscle with SEM (scanning electron microscopy) will be performed to examine in detail mitochondria morphology (Filiano et al, 2002). Any mitochondrial abnormality (if present) will be reported on table 1, along with the patient characteristics:

Table 1: Mitochondrial Data and Motor Impairments in Autistic Children (5-12):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Case # | Age/ Sex | Mitochondrial (Mt) Defect | Mitochondrial Structure | Motor Impairment |
| 1 through 20 | Male/female | If any present | Morphology | H,A,T,R \* |

\*Hypotonia, Apraxia, Toe-walking, Reduced ankle mobility.

*Data Analysis:*

I will be analyzing the prevalence of hypotonia in children with autistic phenotype and whether or not they also present a mitochondrial (Mt) abnormality. Statistical analysis will consist of chi-square tests for categorical data (whether hypotonia is dependent on mitochondrial abnormality) and 95% confidence intervals for reported incidence rates (how often is hypotonia present along with a mitochondrial dysfunction). Correlation coefficient will also be analyzed in order to determine how strong hypotonia correlates with Mt abnormalities. Recorded values will be showed in table 2:

Table 2: Chi square value data

|  |  |  |
| --- | --- | --- |
| Chi square value | Degrees of freedom (Df) n=20 | p- value |
|  | 19 |  |

**Broader implications:**

The discovery of some of Mt disorders in even a small percentage of autistic patients with hypotonia could majorly benefit the individual patient and provide a better understanding of the relationship between such disorders and this motor impairment, and whether or not it is present as a result of a comorbid symptom. Furthermore, proper understanding could lead to determination of effective treatments for mitochondrial dysfunction, which could give the patient a better life quality.

**Time table and spent management:**

Prior to start the research, I must first obtain approval from the Institutional Review Board (IRB) at Florida Gulf Coast University. All the recruited participants for my research will include children exhibiting an autistic phenotype with ages ranging from 5 to 12 years; evaluation by a pediatric neurologist will be required for a proper diagnosis of Autism based on DSM IV (Diagnostic and Statistical Manual of Mental Disorders) criteria (Ming, Brimacombe & Wagner, 2007). Participants will be recruited though advertising at different local medical and/or special educational institutions in the city of Fort Myers including: Gulf Coast Medical Center, The Eden Institute, and Piece by Piece Learning Center among others. Participation will be based upon voluntary desire of the parents/ legal guardians of the children, authorization is required. Recruitment will begin the first week of January 2012 and should be concluded no later than May 12 2012. The research will start on May 12 2012 and it will last approximately 2 months in which I will attain proper data. Completion of research will be expected no later than July 12, 2012.

**Literature Cited:**

- Filiano, J., Goldenthal, M., Rhodes, H., Marin-Garcia, J. 2002.Mitochondrial Dysfunction in Patients with Hypotonia, Epilepsy, Autism, and Developmental Delay: HEADD Syndrome*. Journal of Child Neurology*. 17: 435-439.

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- Ming, X., Brimacombe, M., Wagner, G. 2007. Prevalence of motor impairment in autism spectrum disorders. *Brain and Development*. 29: 565-570.

- Prasad, A. N., Prasad, C. 2011. Genetic Evaluation of the Floppy Infant. *Seminars in Fetal & Neonatal Medicine.* 16: 99-108.

- Rosignol, D.A., Bradstreet, J.J. 2008. Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment. *American Journal of Biochemistry and Biotechnology.* 4 : 208-217.

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- Zecavati, N., Spence, S. 2009. Neurometabolic Disorders and Dysfunction in Autism Spectrum Disorders. *Journal of Pediatric Neurology.* 9: 129-136.

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**GOALS**

* My main goal is to attend medical school and become a physician. I am also interested in the medical field and helping those in need.

**EDUCATION**

* BS: Florida Gulf Coast University, Fort Myers, Florida

 (Anticipated graduation= 2012)

 Major= Biology; Minor= Chemistry

Pre-med track

* High School: Cypress Bay High School (2007)

City: Weston, Florida

Honor Roll (2004-2007)

Associated Courses:

* Evolutionary Biology (2011)
* Biochemistry (2011)
* Immunology (2011)
* Virology (2010)

**EMPLOYMENT**

Currently assisting patients at in emergency room the Gulf Coast Medical Center, Fort Myers. Direct work with patients within the Emergency Room. (2011).

**PROFESSIONAL SOCIETIES and CLUBS**

(2007-2011) National Society of High School Scholars

(2004-2007) National Honor Roll

(2008-2011) National Society of Leadership and Success (Sigma Alpha Phi)

(2004-2011) Pre- med club

**SERVICE**

Volunteer: Currently working as a volunteer at the Gulf Coast Medical Center, Fort Myers. Direct work with patients within the Emergency Room.

Initiated programs:

Pre- med pre professional program.

**AWARDS**

* Pop & Marj Kelly Scholarship

Spring 2011

Amount: $2000

**SCIENTIFIC SKILLS**

-Gel filtration Chromatography

-Cell Culturing

-Gram Staining

-Streak methods for Bacterial Isolations

-Titrations

**Required Equipment:**

**-**SEM (Scanning electron microscope)

-Light Microscope

- Pediatric Neurologist

-20 volunteer participants

-Laboratory elements (microscope slides, pipets, tongs, etc)