The role of retinoic acid related orphan receptor alpha in age-related macular degeneration
BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

THE ROLE OF RETINOIC-ACID-RELATED ORPHAN RECEPTOR ALPHA
IN AGE-RELATED MACULAR DEGENERATION

by

HAI HOANG
B.A., Boston University, 2013

Submitted in partial fulfillment of the
requirements for the degree of
Master of Arts
2015
Approved by

First Reader

Vickery Trinkaus-Randall, Ph.D.
Director, Cell and Molecular Biology Graduate Program
Professor of Biochemistry and Ophthalmology

Second Reader

Neena Haider, Ph.D.
Associate Professor of Ophthalmology
Harvard University, School of Medicine
ACKNOWLEDGMENTS

I would like to acknowledge and thank everyone in the Haider lab group for all of their support and assistance on this project. I would like to thank Dr. Neena Haider for giving me an opportunity to perform my thesis work in her lab, as well as, being an invaluable mentor in guiding me through my project. I want to also thank Dr. Vickery Trinkaus-Randall for being my first reader and assisting on the writing of my thesis.
THE ROLE OF RETINOIC ACID RELATED ORPHAN RECEPTOR ALPHA
IN AGE-RELATED MACULAR DEGENERATION
HAI HOANG
ABSTRACT

Age-related macular degeneration (AMD) is a prevalent cause of vision loss and irreversible blindness that affects more than 11 million Americans. AMD is a multifactorial disease with a number of genetic, demographic, and environmental risk factors. Currently the etiology of AMD is still unclear and there are no effective cure for this devastating disease, but recent studies have demonstrated that RORA is a candidate gene involved in AMD pathophysiology. RORA is a critical regulator of multiple biological processes and has been implicated in various physiological processes including circadian rhythm, lipid metabolism, photoreceptor development, autism, and inflammation. Our current study will explore in depth the role of RORA in AMD. We will look at the effects of RORA in the retina of mice. Localization studies of retinal tissues obtained from mice with a conditional knockout of RORA in epithelial cells showed little effect of RORA on structural cells of the retina. However, there was a decrease in VEGF and TGF-B proteins in RORA knockout. This is an interesting finding because VEGF and TGF-B has an important function in angiogenesis and neovascularization which are pathophysiological effects of AMD. In addition, we will try to identify gene targets of RORA that have also been linked with AMD. By identifying the targets of RORA and discovering how RORA regulates these targets, we hope to better understand the role of RORA in AMD pathophysiology. ChIP-seq and software
analysis of the data was performed to identify all genomic targets of RORA linked with AMD. A number of promising genes were found in both RORA and AMD networks. The next step of this study is to perform quantitative analysis of these genes and how their expression is affected by RORA. Also, we will perform additional conditional RORA knockout models in cone cells and developing retinal cells to further understand the role of RORA in the retina and AMD pathogenesis.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>i</td>
</tr>
<tr>
<td>Copyright Page</td>
<td>ii</td>
</tr>
<tr>
<td>Reader Approval Page</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>iv</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xii</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Age-Related Macular Degeneration (AMD)</td>
<td>1</td>
</tr>
<tr>
<td>Retinoic-Acid-Related Orphan Receptor Alpha (RORA)</td>
<td>5</td>
</tr>
<tr>
<td>Specific Aims and Objective</td>
<td>9</td>
</tr>
<tr>
<td>Methods</td>
<td>10</td>
</tr>
<tr>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>Discussion</td>
<td>30</td>
</tr>
<tr>
<td>List of Journal Abbreviations</td>
<td>36</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Risks of AMD include age, smoking, family history, demographics, and diet:</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>RORA targeted genes that may have associations with AMD</td>
<td>25</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Dry AMD contains the presence of drusen while Wet AMD contains the presence of neovascularization in the retina:</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>RORA is a transcriptional factor that has many physiological functions.</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Genotype analysis shows that all mice used contained mutations of both RORA- floxed and Cdh5-cre:</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>IHC of structural proteins in retina shows no change between RORA knockout and WT</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>IHC of Growth Factors in retina shows decrease expression in RORA knockout:</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>IHC of Photoreceptors in retina shows no difference between RORA mutant and WT</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>IHC of Synaptophysin and Chx10 shows no change between RORA knockout and WT:</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Association between AMD genes and fatty acid metabolic genes that are targets of RORA</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>Association between AMD genes and GPCR genes that</td>
<td>27</td>
</tr>
</tbody>
</table>
are targets of RORA.

10 Association between AMD genes and cell signaling genes that are targets of RORA.

11 Association between AMD genes and transcriptional regulator genes that are targets of RORA.
LIST OF ABBREVIATIONS

AMD ........................................................... Age-Related Macular Degeneration
CHIP .......................................................... Chromatin Immunoprecipitation
CNV ............................................................ Choroidal neovascularization
GCL .................................................................. Ganglion Cell Layer
GFAP ............................................................. Glial Fibrillary Acidic Protein
GPCR .............................................................. G-protein-coupled receptors
IHC .................................................................. Immunohistochemistry
INL .................................................................. Inner Nuclear Layer
IPL .................................................................. Inner Plexiform Layer
NFL .................................................................. Neurofilament Light
ONL .................................................................. Outer Nuclear Layer
OPL .................................................................. Outer Plexiform Layer
RORA ............................................................. Retinoic-Acid-Related Orphan Receptor Alpha
RPE ................................................................. Retinal Pigment Epithelium
TGF-β .............................................................. Transforming Growth Factor Beta
VEGF .............................................................. Vascular Epithelial Growth Factor
INTRODUCTION

**Age-Related Macular Degeneration (AMD)**

Age-related macular degeneration (AMD) is a prevalent cause of vision loss and irreversible blindness that affects more than 11 million Americans according to the BrightFocus Foundation (2013). AMD is a multifactorial disease with a number of genetic, demographic, and environmental risk factors. The strongest risk factors include age, cigarette smoking, diet, and race (Coleman et al., 2008).

<table>
<thead>
<tr>
<th>Risk factors beyond your control</th>
<th>Facts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Adults over age 50</td>
</tr>
<tr>
<td>Family History</td>
<td>AMD runs in families (up to three times the risk)</td>
</tr>
<tr>
<td>Gender</td>
<td>Women are more vulnerable (up to two times the risk)</td>
</tr>
<tr>
<td>Coloring</td>
<td>Those with light colored irises and skin may be more susceptible</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors within your control</th>
<th>Facts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>Low intake of antioxidant vitamins and minerals puts you at higher risk. Canadians 50+ often do not consume Canada Food Guide’s recommendations of 5 to 10 servings of fruits and vegetables daily</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smokers are more susceptible (up to six times the risk)</td>
</tr>
<tr>
<td>Excessive exposure to sunlight</td>
<td>Extended sunlight exposure associated with 10 year incidence of early AMD</td>
</tr>
<tr>
<td>Excessive weight/obesity</td>
<td>Excessive weight increases risk, and a high body mass index increases risk of progression</td>
</tr>
</tbody>
</table>

**Table 1: Risks of AMD include age, smoking, family history, demographics, and diet:** AMD is a multifactorial disease with a number of risk factors. This chart discusses the most common risks of AMD. Obtained from Family Health Magazine, 2012
AMD causes vision impairment through deterioration of the macula, the center of the retina responsible for central vision. This is a slow progressing disease that develops over a number of years, often asymptptomatically in the early stages. (Coleman HR et al., 2008). Early and intermediate stages of AMD are characterized by drusen formation and pigmentary abnormalities, and have a high chance of progression to advanced AMD (Age-Related Eye Disease Study Research Group (AREDSRG), 2005). Drusens are extracellular debris and lipid deposits that localize in between the retinal pigment epithelium (RPE) and Bruch’s membrane. They range in size, color, distribution, and shape. They also, tend to increase with age, and are linked to RPE atrophy and dysfunction (Abdelsalam et al., 1999; Bhutto and Lutty, 2012). RPE cells are essential for vision by being responsible for many complex functions that involve supporting retinal cells (Strauss, 2005). They provide support to retinal cells by providing nutrients, removing waste products, healing wounds, secreting growth factors, and sustaining the choroid and photoreceptors cells (Marshall, 1987; Streilein et al., 2002; Nita et al., 2014).

There are two forms of advanced AMD: dry and wet AMD. Dry AMD is the more common form of AMD, consisting of around 85-90% of total cases. Dry AMD, also known as non-exudative or geographic atrophy, is characterized by atrophy of the cells in the macula responsible for central vision. In dry AMD, drusen accumulates between the retina and choroid which leads to breakdown of retinal pigment epithelial (RPE) cells. RPE atrophy (Geographic atrophy) results in degradation of the photoreceptors leading to decrease in central visual function. Dry AMD progresses slowly, but can manifest into wet AMD in 10% of patients (Sunness et al., 1999).
Wet AMD, also known as exudative or neovascular AMD, occurs in 10% of all AMD cases. Choroidal neovascularization (CNV) is a signature clinical hallmark of wet AMD. CNV is caused by decrease in oxygen and growth factors due to atrophy and dysfunction of the RPE (Bird, 2003). Formation of new blood vessels in the choroid can invade into the retina through Bruch’s membrane and the RPE (Bhutto and Lutty, 2012; Das and McGuire 2003). These vessels are more susceptible to leaking blood and fluids, causing damage and scars, and leading to acute, severe central vision loss (Ding et al., 2008).

Currently there are treatments for some cases of wet AMD, which includes laser treatments and intravitreal injections of vascular epithelial growth factor inhibitors to combat progression of the disease (Keane et al., 2015). In addition, studies have shown taking antioxidant multivitamins and zinc have helped to slow the progress of dry AMD (AREDSRG, 2001). Even with the recent advancement in treating patients with AMD, the etiology and pathogenesis of AMD is poorly understood. Currently, there is no known cure of AMD or effective prevention of the disease. Therefore it is important to discover new methods that can help detect early manifestation of AMD. Due to the progressive nature of the disease, it is beneficial to understand the development and progression of the disease to provide better treatments.
Figure 1: Dry AMD contains the presence of drusen while wet AMD contains the presence of neovascularization in the retina: This figure illustrates the pathophysiological findings of advanced AMD. Dry AMD is characterized by drusen formation causing RPE dysfunction which leads to retinal cells degeneration. Wet AMD is characterized by choroidal neovascularization, which can cause leaking blood and fluid into the retina. Figure obtained from BrightFocus foundation, 2013.
Retinoic-Acid-Related Orphan Receptor Alpha (RORA)

Recent studies on the interaction between genetic and environmental factors in the disease have implicated a number of genes in the pathogenesis of AMD (Scholl et al., 2007). A promising study, performed previously in this research lab, have linked retinoic-acid-related orphan receptor alpha (RORA or NR1F1) to the pathogenesis of AMD (Silveira et al., 2009). RORA is a member of the nuclear receptor superfamily. Nuclear receptors are a type of transcription factor because they can directly bind to DNA to regulate gene expression. Many nuclear receptors have ligands that cause conformational change once bound. This conformational change activates the nuclear receptor enabling to interact with specific response elements, and subsequently alter gene expression (Olefsky, 2001). Nuclear receptors are a diverse family, with a total of 48 known human nuclear receptors (Zhang et al., 2004). They are involved in the etiology of many diseases and functions in almost all physiological processes including development and homeostasis. Currently, it is a promising area of research, which has major implications in physiology, therapeutic treatments, and drug development.

Orphan receptors are a group of nuclear receptors that currently do not have any known ligands. RORA is an orphan receptor since its endogenous ligand is not fully agreed upon. Many recent studies have identified potential ligands that can modulate this NR to identify/understand its physiological role, but none has been fully agreed upon (Solt et al., 2012). RORA is a critical regulator of multiple biological processes and has been implicated in various physiological processes including circadian rhythm, lipid metabolism, photoreceptor development, autism, bone morphogenesis, and inflammation.
(Jetten et al., 2009). Its expression is distributed widely through several organs and tissues including brain, liver, testis, skin, and bone (Giguere et al., 1994).

![Figure 1: RORA is a transcriptional factor that has many physiological functions.](image)

This diagram illustrates how RORA binds to its response element (AGGTCA preceded by 6 A/T) to affect the transcription of many target genes. RORA has a wide variety of gene targets in almost all physiological functions. It is also been implicated in a number of diseases, which makes it an ideal target for novel therapeutic treatments. Obtained from National Institute of Environmental Health Sciences, 2011

RORA has a direct role in the retina, specifically in photoreceptor development. RORA expression is found in GCL, INL, and cone photoreceptors in mouse adult retinas.
It is also highly expressed throughout the retina during embryonic development where it is involved in retinal cone photoreceptor cells development (Fujieda et al., 2009).

RORA has been found to have a role in circadian rhythms. Circadian rhythms have an essential role in regulation of almost all normal physiology processes and behavior. Many cells contain its own circadian oscillator that operates in an independent manner. However, all of the cells are coordinated by the master circadian clock located in the brain (Ko and Takahashi, 2006). The master clock is in turn regulated in part by the light-dark input by the visual photoreceptor systems in the retina. A number of genes (BMAL1, CLOCK, PER, CRY) that are essential in the molecular circuitry of circadian clock are regulated by RORA through positive and negative feedback loop pathways (Isojima 2003). Circadian rhythms have a role in metabolism thus implicating RORA to have an intermediate role to couple circadian clock with metabolism. Fluctuations in daily levels of glucose, lipids, insulin, and other important molecules in metabolism suggest that the circadian cycle plays an important role in regulation of metabolic pathways. Disruption of this cycle can result in manifestation of metabolic disease such as obesity, diabetes, and cardiovascular defects which all have AMD risk associations (Duez and Staels 2008).

In addition, RORA has been directly implicated in cellular metabolism especially lipid and cholesterol metabolism. Reduced expression of cholesterol transporters (ABCA1, ABCA8, APOA1) is a proposed mechanism in which RORA affects cholesterol and lipoproteins levels (Lau et al., 2008). Also, cholesterol has been reported to be a potential natural endogenous ligand of RORA (Kallen el al., 2002). This is a
possible pathway in which RORA can affect AMD, since lipid metabolism plays a role in formation of drusen and angiogenesis.

Angiogenesis is one of the clinical manifestations of advanced neovascular AMD. Low oxygen levels play an important role in inducing angiogenesis, vasculogenesis, and tumorigenesis (Besnard et al., 2001). Studies have shown that RORA is upregulated in not only hypoxic conditions, but also under oxidative stress. Oxidative stress has also been implicated in AMD due to its pro-inflammatory responses that leads to retinal damage (Beatty et al., 2000). AMD development has been strongly linked to the immune system and inflammatory pathway (Klein et al., 2005). This is another pathway where RORA and AMD are linked.
SPECIFIC AIMS AND OBJECTIVE

Age-related macular degeneration is a devastating disease that affects millions of people worldwide, yet the etiology and pathogenesis of AMD is not fully understood. A recent study by Jun et al. (2011) has demonstrated RORA as a candidate gene involved in AMD pathophysiology. RORA has numerous physiological functions which are common in AMD pathology. Our current study will further explore the role of RORA in AMD. We will look at the effects of RORA in the retina of mice by immunohistochemistry. Antibodies against retinal cell-specific markers and proteins of interest will be used to detect their expression in the retina. In addition, we will try to identify gene targets of RORA that have also been linked with AMD. By identifying the targets of RORA and discovering how RORA regulates these targets, we hope to better understand the role of RORA in AMD pathophysiology. This study is an important step to understanding this complex disease and is necessary to form novel therapeutic treatments in the future.
METHODS

MICE

All mice were bred and maintained under standard conditions at Schepens Eye Research Institute. Rora(flox/flox) were crossed with Cdh5-Cre to produce offsprings with both mutations (Rora(flox/flox) x Cdh5-Cre). Tissues were harvested from control, wild type C57BL6/J (WT) mice, Rora(flox/flox) mice, and Cdh5-cre.

GENOTYPING

Mice were genotyped by PCR to ensure each mutation were present. Tail samples were collected and DNA was extracted for genotyping using the isopropanol method. Tail samples were submerged in a solution of 250µl tail buffer and 7µl proteinase K, and incubated for 2 hours at 65°C, vortexing every 10 minutes. 200µl of 5M ammonium acetate was added to the solution, and then incubated on ice for 15 minutes. Solution was centrifuged for 20 minutes at 13,000 RPM. Afterwards, the supernatant was transferred to a new tube containing 750µl isopropanol, and then centrifuged for 10 minutes at 16,000 RPM. The supernatant was removed, and 1 ml of 70% ethanol was added. Solution was centrifuged for 5 minutes at 16,000 rpm. Supernatant was discarded, and pellet was dried for 5 minutes on heat block at 55 °C. 30µl of DEPC treated water was added, and tube was incubated for 10 min at 55°C. The concentration of DNA extracted was measured using Thermo Scientific NanoDrop 2000 Spectrophotometer.

PCR master mix component (per sample): 10x PCR buffer (1µl) (Roche Life Sciences), 40mM dNTP (0.20 µl) (Roche Life Sciences), 10µM forward primer (0.25 µl),
10µM reverse primer (0.25µl), Taq Polymerase 5U/µl (0.1 µl) (Roche Life Sciences), DNA sample 50 ng/µl (0.7µl). Final reaction = 10 µl. The following primers were used:

RORA-flox (Forward, 5’- TCT GAA TCC ACC ATA CTT CC -3’, Reverse, 5’- AGG TCT GCC ACG TTA TCT G -3’) (Eurofins MWG Operon), Cdh-Cre (Forward, 5’- GTG AAA CAG CAT TGC TGT CAC TT -3’, Reverse, 5’- GCG GTC TGG CAG TAA AAA CTA TC -3’) (Eurofins MWG Operon).

All reactions were performed in 200µl PCR tubes, and were run in a Bio-Rad C1000 Touch Thermal Cycler. Cycle parameters for Rora-floxed: 1) 95°C for 3 minutes, 2) 95°C for 15 sec, 3) 65°C for 30 seconds, decrease 1°C/cycle, 4) 72°C for 40 seconds, 5) Go to step 2 for 10 cycles, 6) 95°C for 15 seconds, 7) 55°C for 30 seconds, 8) 72°C for 40 seconds, 9) Go to step 6 for 30 cycles, 10) 4°C hold until refrigerate product. Cycle parameters for Cdh-cre: 1) 95°C for 3 minutes, 2) 95°C for 30 seconds, 3) 51.7°C for 1 minute, 4) 72°C for 1 minute, 5) Repeat steps 2-4 for 34 cycles, 6) 72°C for 2 minutes, 8) 12°C hold. Products were analyzed on 2% agarose gel with ethidium bromide staining. Product size: 323 bp WT RORA, 394 bp mutant RORA-flox, 100 bp Cre product.

IMMUNOHISTOCHEMISTRY

Eyes from wild type B6 mice, Rora-floxed, Cdh-Cre, and Rora/Cdh mice were collected for paraffin embedding. Eyes were stored in Methanol: Acetic Acid (3:1) fixative or 4% Paraformaldehyde (PFA) fixative overnight at 4°C. Eyes were then placed in embedding cartridges with the following solution: 70% ethanol for 2 hours, 95%
ethanol for 2 hours, 100% ethanol for 2 hours, xylene for 1 hour, paraffin overnight.

Tissue blocks were sectioned at 5µm and collected on coated slides.

Deparaffinization of tissue sections were performed using xylene twice for 5 minutes each. Afterwards, tissues were dehydrated in decreasing ethanol concentration (100%, 95%, 70%; twice for 5 minutes each). PFA sections were heat treated with citric acid before incubating with primary antibodies. Citric acid treatment of slides involved microwaving slides for 60 seconds submerged in 10mM sodium citrate buffer (pH 6.0). Sections were then blocked with 2% horse serum diluted in PBS for 45 minutes at room temperature to prevent non-specific binding of primary antibodies. Tissue sections were incubated with primary antibodies at 4°C overnight. Secondary antibodies were incubated for 45 minutes at room temperature. Cell nuclei were counter stained with 4',6-diamidino-2-phenylindole (DAPI, diluted 1:200). Finally, sections were mounted with glass cover slips using Vectashield (Vector Lab, H-1200). Fluorescence images were examined by confocal laser scanning microscope (Leica TCS SP8).

The following primary antibodies were used: mouse polyclonal antibodies to Vascular Epithelial Growth Factor (VEGF, Abcam, Ab1316), rabbit polyclonal antibodies to Transforming Growth Factor Beta (TGF-β, Abcam Ab66043), rabbit polyclonal antibodies to Glial Fibrillary Acidic Protein (GFAP, EMD Millipore, Ab5804), sheep polyclonal antibodies to Chx10 (CHEMICO International, Ab9014), mouse polyclonal antibodies to Calbindin D-28K (Swant, Cb300), rabbit polyclonal antibodies to Parvalbumin (Abcam, Ab11427), rabbit polyclonal antibodies to Collagen IV (Abcam, ab6586), rabbit polyclonal antibodies to Beta-Tubulin III (Sigma-Aldrich,
T2200), rabbit polyclonal antibodies to Neurofilament Light (NFL, EMD Millipore, Ab9568), rabbit polyclonal antibodies to Synaptophysin (Abcam, ab52636), rabbit polyclonal antibodies to Protein Kinase C alpha (PKC-α, Abcam, Ab31), goat polyclonal antibodies to OPN1SW (Santa Cruz Biotechnology, SC-14363), mouse polyclonal antibodies to Rhodopsin (EMD Millipore, MAB5316), and rabbit polyclonal antibodies to Red/Green Opsin (EMD Millipore, ab5405). Dilutions of primary antibodies were as followed: VEGF 1:100, TGF-β 1:100, GFAP 1:200, Chx10 1:200, Calbindin 1:200, Parvalbumin 1:200, Collagen IV 1:400, β-Tubulin III 1:400, NFL 1:200, Synaptophysin 1:200, PKC-α 1:200, OPN1SW 1:200, Rhodopsin 1:200, Red/Green Opsin 1:200. The following secondary antibodies were used: Alexa Fleur 488 goat anti-rabbit IgG (Life Technologies, A11008), Alexa Fleur 488 goat anti-mouse IgG (Life Technologies, A11001), Alexa Fleur 555 donkey anti-sheep IgG (Life Technologies, A21436). All secondary antibodies were diluted 1:400. Both primary and secondary antibodies were diluted using 2% horse serum.

**CHROMOTIN IMMUNOPRECIPITATION**

Chromatic immunoprecipitation (ChiP) was performed as previously described (Haider et al., 2009). 8 retinas from B6 mice were obtained. The retina was disrupted using a pestle and mortar. 37% formaldehyde was added to the tissue for 60 minutes at room temperature in order to crosslink proteins with DNA. The samples were then sonicated to shear the DNA. Samples were sonicated with 10 pulses for 1 second. This was done 20 times with 10-second pause between pulses. RORA antibody (1μg) was
added to bind to cross-linked protein/DNA. Samples were incubated overnight at 4°C on a rotating platform. Solution went through a number of washes in order to obtain fragments of interest. The remaining fragments were reverse cross-linked by incubating with 200mM NaCl and 10mg of Protinase K (to remove proteins) for 5 hours at 65°C. Qiagen purification kit was used to purify the DNA samples and concentrations were measured Thermo Scientific NanoDrop 2000 Spectrophotometer. Samples were sent to for sequencing.

**PATHWAY ANALYSIS**

Data from the ChIP assay was used to find statistically significant genes of interest regulated or interacted with RORA. Data were analyzed using Ingenuity Pathway Analysis (IPA, Ingenuity Systems, [www.ingenuity.com](http://www.ingenuity.com)) as described in a previous study (Jelcick et al., 2011). Networks were generated based on their connectivity. Gene identifiers and statistically significant expression values were uploaded into Ingenuity. Default cutoffs were set to identify genes whose expression was significantly differentially regulated and overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks were algorithmically generated based on their connectivity. Genes or gene products in the networks are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). Dashed edges represent a weaker relationship than solid edges. All edges are supported by at least 1 reference from the literature, from a textbook,
or from canonical information stored in the Ingenuity Pathways Knowledge Base. Nodes are displayed using various shapes that represent the functional class of the gene product.
RESULTS

MICE GENOTYPING

A mouse model was produced with a conditional knockout of RORA in endothelial cells by using Cre-lox recombination technology. Rora (Floxed/floxed) was crossed with Cdh5-cre to create a new mouse strain with both mutation: RORA (floxed/floxed) x Cdh5-cre. This strain is a conditional knockout of RORA in only cells that expresses VE-Cadherin which are endothelial cells.

Genotyping was performed to ensure that the mice have both mutations (Figure 3). Positive control, negative control, and B6 control samples were used to ensure the correct mutations were analyzed. RORA-floxed product size was 394bp and RORA WT product size was 323bp. Cdh5-Cre product size was 100bp. The following offspring of crossed RORA-floxed and Cdh-cre were shown to have both mutations: 79, 80, 81, 82, 83, 84, 85, 103, 104, 105 112, 113, 114, 115. The following did not have Rora-flox mutations but contained only Cdh-cre: 100, 101, 102, 106, 107, 116.
Figure 2: Genotype analysis shows that all mice used contained mutations of both RORA- floxed and Cdh5-cre: Mice were genotyped to determine if mutations were present in genome. RORA mutant band is 394 bp and WT RORA is 323 bp. Cdh5-cre product is 100bp. The following animals are homozygous for RORA mutant as well as Cdh5-cre: 79-85, 103-107, 112-115.

IMMUNOHISTOCHEMISTRY

Structural proteins (GFAP, NFL, Collagen IV, β-tubulin III):

Glial fibrillary acidic protein is a type III intermediate filament and functions to maintain cell shape and structure. It is a major constituent in astrocytes. It serves as a cell specific marker to distinguish between astrocytes from other glial cells. Astrocytes are found in the nerve fiber layer (NFL) and ganglion cell layer (GCL) where they provide
support for the ganglion cells. GFAP immunoreactivity was confined to the GCL in normal B6 mice as well as in the RORA KO (Figure 4).

Neurofilament light (NFL) are intermediate filament that are major components of the neuronal cytoskeleton. It is found primarily in the axons of neurons where its function is to provide structural support for axon and regulate axon diameter and shape. Immunoreactivity of NFL is found mainly in ganglion cells and horizontal cells in the GCL and OPL (Figure 4).

Collagen IV is a type of collagen that is a major constituent in the basement membrane of tissues. Immunoreactivity of collagen IV is seen in the GCL, IPL, OPL, and RPE (Figure 4).

Beta Tubulin III (β-tubulin III) is a major component of microtubules that is found only in neurons. It is a marker to distinguish between neurons and glial cells in samples of neural tissues. The functions of β-tubulin III include axon guidance and maintenance. Immunoreactivity of β-tubulin III is seen in the IPL and OPL (Figure 4).
Figure 4: IHC of structural proteins in retina shows no change between RORA knockout and WT: GFAP, Collagen IV, NFL all has similar staining across all four mice. GFAP had staining in GCL and OPL. Collagen IV had staining at GCL, IPL, OPL, and RPE. NFL had staining at GCL, IPL, and OPL. Scale bar: 100 µm. Nuclei of cells are stained blue and proteins of interest are stained green (GFAP, Collagen IV, NFL, B-Tubulin III)

Growth factors (VEGF, TGF-β):

Vascular epithelial growth factor (VEGF) is responsible for stimulating angiogenesis, epithelial cell growth, and vasculogenesis. VEGF plays a role in wet AMD
and diabetic retinopathy where there is new blood vessels formation in the retina causing loss in visual acuity. VEGF is expressed in the retina by Muller cells, endothelial cells, ganglion cells, RPE cells, and astrocytes. Immunoreactivity of VEGF is seen in the GCL and OPL. There seems to be an absence of VEGF staining in the knockout strain, and interestingly a decrease in the Cdh5-cre mice (Figure 5).

Transforming growth factor beta (TGF-β) is a cytokine whose many functions include control of cell growth, cell proliferation, cell differentiation, and apoptosis. It is also linked with vascular barrier function, endothelial permeability, and angiogenesis which suggests it has a role in neovascularization (Walsh et al., 2009). It is expressed in ganglion cells, endothelial cells, and photoreceptors. Immunoreactivity of TGF-β is seen in the GCL and OPL in normal WT mice. There seems to be a decrease of staining in the knockout mice (Figure 5).

**Figure 5:** IHC of Growth Factors in retina shows decrease expression in RORA knockout: VEGF and TGF-B is involved in angiogenesis and cell growth.
Immunoreactivity of both VEGF and TGF-B is low in Rora-floxed x Cdh5-Cre (D,F) compared to WT (A-C, E-G). Scale bar: 100 µm. Nuclei of cells are stained blue and proteins of interest are stained green (VEGF, TGF-B)

**Retinal Cell Specific Markers (Calbindin, Parvalbumin):**

Calbindin D-28K is a calcium binding protein. It is a retinal cell specific marker for horizontal cells in the retina. Parvalbumin is a calcium binding protein. It is a retinal cell specific marker for amacrine cells in the INL. IHC results show similar immunoreactivity in all mouse tissues for calbindin and parvalbumin.

**Photoreceptors (Blue opsin, Green opsin, Rhodopsin):**

Blue opsin, green opsin, and rhodopsin are markers for photoreceptor cells in the retina. Blue opsin are found in S-cone photoreceptor, green opsin are found in M-cone photoreceptor, and rhodopsin are found in rod photoreceptors. They are all expressed in the photoreceptor layer staining the different photoreceptors. All of the immunostaining were similar in the four different strains of mice (Figure 6).
**Figure 6: IHC of Photoreceptors in retina shows no difference between RORA mutant and WT:** S-cone, M-cone, and rod cells were stained by blue opsin, green opsin, and rhodopsin Ab respectively. All immunoreactivity of each protein were similar in both WT and mutant (A-L). Scale bar: 100 µm. Nuclei of cells are stained blue and proteins of interest are stained green (Blue opsin, Green opsin, Rhodopsin).

**Bipolar cells (Chx10, Synaptophysin, PKCa):**

Synaptophysin is a protein found in neurons involved in synaptic transmission. Its function is currently unknown but it is a part of the synaptic vesicle complex. It is found in both the inner plexiform layer (IPL) and outer plexiform layer (OPL) since this is where synaptic transmission occurs in the retina. Synapses of photoreceptors with bipolar
cells and horizontal cells occur in the OPL and Synapses of bipolar cells with ganglion cells and amacrine cells in the IPL. Immunoreactivity of synaptophysin is seen in the IPL and OPL as expected in WT, Rora-floxed, Cdh5-cre, and Rora-floxed x Cdh5-cre (Figure 7).

Protein Kinase C alpha (PKCα) is a protein kinase that has many physiological roles and targets. It has been suggested that PKCα regulates bipolar cells signal transduction (Ruether et al., 2009). In the retina, it is mainly expressed in bipolar cells which are found in the inner nuclear layer (INL), OPL, and IPL.

Chx10 is a protein that is involved in retina development. It is necessary in the developing retina where it helps retinal progenitor cells differentiate into mature cells. Chx10 expression is high in the developing retina, but in mature retina, it is confined to bipolar cells expressed at low levels. Immunoreactivity of Chx10 is seen in the INL as expected in WT, Rora-floxed, Cdh5-cre, and Rora-floxed x Cdh5-cre (Figure 7).
Figure 7: IHC of Synaptophysin and Chx10 shows no change between RORA knockout and WT: Chx10 and Synaptophysin immunoreactivity were similar between WT and Rora-floxed x VE-Cdh-Cre tissues. Scale bar: 100 µm. Nuclei of cells are stained blue and proteins of interest are stained red (Synaptophysin, Chx10).

CHROMATIN IMMUNOPRECIPITATION – SEQUENCING (CHIP-SEQ)

Chromatin immunoprecipitation with massively parallel sequencing is very useful in mapping DNA-protein interactions on a genomic-wide level. ChiP assay is an effective assay for transcription factors because binding of TF are very sequence specific, which leads to very localized ChiP-seq signals in the genome. ChIP data reveals numerous gene targets of RORA. 1150 genes were found to be statistical significant binding targets of RORA.

INGENUITY: INTEGRATED PATHWAY ANALYSIS

AMD genes and RORA pathway genes were inputted into Ingenuity IPA to analyze any known associations between these genes. Multiple pathways were found and linked to AMD (Figures 8-11). These genes were then compared to the list of RORA targets from the ChIP data. A list of genes appearing in both lists are our genes of interests which are RORA targeted genes that are linked with AMD (Table 2).
<table>
<thead>
<tr>
<th>AMD Genes</th>
<th>Proteins that genes code for</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>Complement factor H</td>
</tr>
<tr>
<td>ELOVL4</td>
<td>Elongation of very long chain fatty acids protein 4</td>
</tr>
<tr>
<td>FBLN5</td>
<td>Fibulin-5</td>
</tr>
<tr>
<td>FLT1</td>
<td>Vascular epithelial growth factor receptor 1</td>
</tr>
<tr>
<td>MPDZ</td>
<td>Multiple PDZ protein</td>
</tr>
<tr>
<td>PLEKHA1</td>
<td>Pleckstrin homology domain-containing family A member 1</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GPCR geness</th>
<th>Proteins that genes code for</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD3</td>
<td>D3 dopamine receptor</td>
</tr>
<tr>
<td>RAMP2</td>
<td>Receptor activity monitoring protein 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transcriptional Regulator genes</th>
<th>Proteins that genes code for</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLDN3</td>
<td>Claudin 3</td>
</tr>
<tr>
<td>CNPY2</td>
<td>Canopy FGF Signaling Regulator 2</td>
</tr>
<tr>
<td>HIST1H2AD</td>
<td>Histone H2A type 1-D</td>
</tr>
<tr>
<td>MYCBP</td>
<td>C-Myc binding protein</td>
</tr>
<tr>
<td>RBM22</td>
<td>RNA binding motif protein 22</td>
</tr>
<tr>
<td>RNF113A</td>
<td>Ring finger protein 113A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell signaling genes</th>
<th>Proteins that genes code for</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARHGAP19</td>
<td>Rho GTPase-activating protein 19</td>
</tr>
<tr>
<td>EXOSC4</td>
<td>Exosome component 4</td>
</tr>
<tr>
<td>PSD</td>
<td>Pleckstrin and Sec7 Domain Containing</td>
</tr>
<tr>
<td>RAB40C</td>
<td>Ras-related protein Rab-40c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fatty Acid Metabolism genes</th>
<th>Proteins that genes code for</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP1</td>
<td>Insulin-like growth factor binding protein 1</td>
</tr>
<tr>
<td>N-cor</td>
<td>Nuclear receptor co-repressor 1</td>
</tr>
<tr>
<td>VLDLR</td>
<td>Very low density lipoprotein receptor</td>
</tr>
<tr>
<td>ZBTB9</td>
<td>Zinc finger and BTB domain containing 3</td>
</tr>
</tbody>
</table>

Table 2: RORA targeted genes that may have associations with AMD: Genes from ChIP data was compared with Ingenuity pathway analysis to determine genes that are common. These genes are targets of RORA and genes that are also linked with AMD.
Figure 8: Association between AMD genes and fatty acid metabolic genes that are targets of RORA.: AMD genes are purple while fatty acid metabolism genes are orange. Lines and arrows show associations between the genes. Figure was produced by Ingenuity IPA. Genes of interest include: N-cor, PPARG, Smad, VLDLR, LDLR, IGFBP1, RARRES2, APOE3.
Figure 9: Association between AMD genes and GPCR genes that are targets of RORA. AMD genes are purple while GPCR genes are green. Lines and arrows show associations between the genes. Figure was produced by Ingenuity IPA. Genes of interests include: Beta arrestin, GPCR, RAMP2, DRD3
Figure 10: Association between AMD genes and cell signaling genes that are targets of RORA. AMD genes are purple while cell signaling genes are light blue. Lines and arrows show associations between the genes. Figure was produced by Ingenuity IPA. Genes of interest include ERK1/2, RAB40C, CLDN, PRKAC
Figure 11: Association between AMD genes and transcriptional regulator genes that are targets of RORA. AMD genes are purple while transcriptional regulator genes are red. Lines and arrows show associations between the genes. Figure was produced by Ingenuity IPA. Genes of interest include HIST1H2AD, PINKX1, 60S ribosomal subunit, EIF1B, PKA, MYCBP.
DISCUSSION

A conditional knockout model was used to explore the effects of RORA in the retina. In the knockout model, RORA was conditionally knocked out in endothelial cells. The retinal pigment epithelium is a layer in the retina that supports, nourishes, and protects the retinal cells so that they can maintain normal visual function. Since the dysfunction of this layer of cells is critical in the development of AMD, we will study the effect of RORA knockout in these types of cells.

Immunohistochemistry was performed to detect the levels of expression of various proteins and cell markers in the retina. Immunostaining of GFAP, NFL, Collagen IV, and β-Tubulin III were checked to see if RORA had any effect on structural proteins and structural changes in the retina. In addition, cell specific markers were also checked to see any changes in the different cells in the retina. Blue opsin, green opsin, rhodopsin, parvalbumin, and calbindin stains S-cone cells, M-cone cells, rod cells, amacrine cells, and horizontal cells respectively. IHC results shows that there is not much difference in levels of expression in either WT or RORA knockout (Figures 4, 6). In addition, we checked synaptophysin because it is important in normal neuron functions through its involvement in synaptic transmission. Synaptophysin expression was normal (Figure 7). Since RORA and Chx10 both are important in retinal cell differentiation and development, levels of Chx10 expression were also investigated which resulted in similar expression in WT and knockout (Figure 7).

VEGF and TGF-β are growth factors whose functions include angiogenesis, vascular permeability, cell growth, cell differentiation, and vasculogenesis. IHC of VEGF
and TGF-β shows decrease levels of expression in RORA knockout compared to WT (Figure 5). This is an interesting finding due to the fact that neovascularization in the retina often leads to severe acute visual loss in wet AMD. CNV is a significant clinical manifestation of neovascular AMD, and recent studies have suggested the upregulation of angiogenic factors (VEGF, TGFβ, angiostatin) to be involved in the formation of CNV (Zhang, 2007). In addition, a previous study suggested the presence of TGF-β in AMD pathophysiology (Silveira et al., 2010). Also, current treatment for wet AMD involves injections VEGF inhibitors to delay the progression of the disease (Kovach et al., 2012). Additional conformational experiments will be done to confirm that these changes are noted.

Another important part of this study is to investigate the vast RORA pathway and how it is linked to AMD pathogenesis. Since RORA is a nuclear receptor that has been implicated in a variety of physiological functions (Solt et al., 2012), it will be invaluable to identify the gene targets of RORA. Using the ChIP-seq technique, we were able to determine the sequences of DNA that RORA binds to in vivo. Then, with software analysis, a list of statistically significant RORA gene targets was determined. Comparing this lists with a list of commonly known AMD genes, we determined a number of genes to further investigate (Table 2, Figures 8-11). Our results shows that AMD may have some associations with genes in a number of pathways including fatty acid metabolism, G-protein-coupled receptors, transcriptional regulation, and cell signaling.

Fatty acid metabolism has been shown to be involved in AMD pathology and development. Cholesterol and lipids are major components of drusens, whose presence is
a clinical hallmark of AMD. In addition, recent studies have linked AMD risks to ATP binding cassette transporters, which are a major regulator of cholesterol and phospholipids (Allikmets, 2000). Our results have shown that IGFBP1, VLDLR, NCOR, and ZBTB9 are linked with AMD and are potential gene targets of RORA (Table 2, Figure 8). IGFBP1 is a binding protein that binds to IGF to extend its half-life to regulate its cellular availability (Juul, 2003). IGF has been shown to regulate metabolic processes and cell growth and development (Thissen et al., 1994), and recent studies have suggested that IGF1 and IGFBP1 have a role in AMD pathogenesis through the inflammatory pathway (Chiu et al., 2011). VLDLR is a transmembrane protein receptor that is involved in cholesterol uptake and fatty acid metabolism. Studies of VLDLR have shown in the absence of VLDLR, angiogenesis increases in the retina, a major complication in wet AMD (Hu et al., 2008; Jiang et al. 2009).

G-protein coupled receptors (GPCR) are the largest class of receptors and have roles in many physiological processes, including fatty metabolism, cell signaling, and transcriptional regulation. One example of a GPCR in the retina is rhodopsin, which is found in photoreceptors and is responsible for light transduction (Hamm, 2000). GPCR has been linked to the production of oxidation in the retina (Chen et al., 2012). Oxidative stress contributes to photoreceptor degeneration and damage to the retina, especially the RPE, which has implications in the development of AMD (Beatty et al., 2000). In addition, one current treatment for dry AMD is taking antioxidant multivitamins, which are shown to slow progression of the disease (AREDSRG, 2001).
Our data suggests that Rab40c and ARHGAP19 is targeted by RORA and is associated with the AMD pathway (Table 2, Figure 10). Rab40c and ARHGAP19 are small GTPase, which also links it to the GPCR pathway. Recent studies have shown that Rab40c is linked with formation and regulation of lipid droplets, which stores lipids and cholesterol (Tan et al., 2013). Lipid droplet dysfunction has a role in many metabolic diseases such as obesity and diabetes, which share risk factors for AMD (Greenberg et al., 2011). By linking AMD and RORA we can better understand the pathway in which AMD progresses and develops.

Cell signaling is an important system that controls cellular activities and coordinates cellular actions. One example is that TGF-β downstream pathway includes many extracellular signal-regulated kinases (ERK/MAP) targets in order to induce its effects on the cells (IKushima and Miyazono, 2010). In addition, an important signaling pathway of AMD involves the immune system. Immune-mediated responses and inflammatory processes has a role in drusen formation and promoting CNV in the retina (Hageman et al., 2001). Chemokines (IL-6, CCL2, CCR2, CX3CR1, TNF), cell signaling molecules in immune processes, have been linked to AMD since they have shown damaging effects on Bruch membrane, RPE, and the retina (Ambati, Atkinson, and Gelfand, 2013). Also, AMD risk has been strongly linked with the complement cascade system; drusens contain almost all the complement proteins. Genetic variations in complement genes (CFH, C3) have shown an increase risk for the disease (Klein et al., 2005).
Transcriptional regulation affects gene and protein expression. RORA is a transcriptional factor that regulates many different gene targets (Jetten et al., 2009), and it would be expected that RORA also have some targets that has a function in transcription. This will be important in elucidating a pathway on how RORA has an effect in AMD pathogenesis.

The next step of this study is to further explore the targeted RORA genes that have been linked to AMD. Quantitative real-time PCR will be performed to investigate how these gene expression levels are affected in the conditional RORA knockout mice. Genes that show altered expression levels will be promising targets to further analyze. In addition, we will further investigate the role of RORA in the retina and in the etiology of AMD. Future studies will explore the effect of RORA in the retina of conditional knockout of RORA in cone cells and Chx10. By exploring these knockout models, we will gain a better understanding of the role of RORA in the photoreceptors and the developing retina.

AMD is the one of the most prevalent causes of decrease in visual acuity and irreversible blindness in the world. AMD is a complex genetic disease that has a number of genes and environmental factors that is implicated in its etiology. There is currently no cure for AMD, but because of its negative effect on quality of life, high economic costs, and public health implications, there is an increasing urgency for research in this area (Day et al., 2011; Soubrane et al., 2007). RORA has been linked with AMD pathogenesis, and it is a promising area to explore to because of its effects in the retina and numerous physiological pathways. This study is one small step towards elucidating
both the etiology of AMD and the role of RORA in AMD. This will be very beneficial to developing novel treatments and therapeutic drugs targets to cure this disease in the future.
# LIST OF JOURNAL ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arch Ophthalmol</td>
<td>Archives of Ophthalmology</td>
</tr>
<tr>
<td>Circ Res</td>
<td>Circulation Research</td>
</tr>
<tr>
<td>Diab Vasc Dis Res</td>
<td>Diabetes &amp; Vascular Disease Research</td>
</tr>
<tr>
<td>Endocr Rev</td>
<td>Endocrine Reviews</td>
</tr>
<tr>
<td>Genes Dev</td>
<td>Genes and Development</td>
</tr>
<tr>
<td>Growth Horm IGF Res</td>
<td>Growth Hormone &amp; IGF Research</td>
</tr>
<tr>
<td>Hum Mol Genet</td>
<td>Human Molecular Genetics</td>
</tr>
<tr>
<td>Invest Ophthlmol Vis Sci</td>
<td>Investigative Ophthalmology &amp; Visual Science</td>
</tr>
<tr>
<td>J Biol Chem</td>
<td>Journal of Biological Chemistry</td>
</tr>
<tr>
<td>J Neurochem</td>
<td>Journal of Neurochemistry</td>
</tr>
<tr>
<td>Nat Struct Mol Biol</td>
<td>Nature Structural &amp; Molecular Biology</td>
</tr>
<tr>
<td>Physiol Rev</td>
<td>Physiological Reviews</td>
</tr>
<tr>
<td>Prog Retin Eye Res</td>
<td>Progress in Retinal and Eye Research</td>
</tr>
<tr>
<td>Surv Ophthalmol</td>
<td>Survey of Ophthalmology</td>
</tr>
<tr>
<td>Vision Res</td>
<td>Vision Research</td>
</tr>
</tbody>
</table>
REFERENCES


CURRICULUM VITAE

Hai Hoang
Hhoang6959@gmail.com
YOB: 1991
203-522-0440
Permanent Address:
1842 Main Street
Stratford, CT 06615

Education:  Boston University School of Medicine – Boston, MA
M.A., Medical Science, Expected May 2015

    Boston University – Boston, MA
B.A., Biology, conc. in Molecular, Cell Biology and Genetics, May 2013
B.A., Economics, May 2013

Research Experience:
    Schepens Eye Research Institute – Boston, MA
Research Intern – September 2014 - present

    Worked on an independent project investigating the role of RORA in macular degeneration disease. Utilized a variety of lab techniques such as immunochemistry, qPCR, genetic pathway analysis, and genotyping. Completed my master thesis in this lab.

    Harvard School of Public Health, GCD – Boston, MA
Research Intern – January 2012 – May 2013

    Assisted lab members with their projects and learning advanced laboratory techniques such as flow cytometry, ELISA, and tissue culture. Worked alongside a post-doc doing supplementary projects to assist with his thesis. Assisted lab manager with lab duties such as making buffers, ordering supplies, stocking benches and hood. Personal project involved examining the pathway of inflammation regulation and more specifically how mitochondria are involved in this pathway.

    St. Vincent Medical Center, Emergency Department – Bridgeport, CT
Research Associate – May 2011 – August 2011

    Surveyed and enrolled patients in a tobacco cessation study. Interacted with patients and hospital staff in a friendly and proficient manner.
Yale School of Medicine, Dept. of Infectious Disease – New Haven, CT
Research Assistant – May 2010 - August 2010

Assisted research with a post-doctorate at Yale who was conducting research on HIV treatment through the use of siRNA. Utilized lab techniques such as gel analysis, protein purification, amplification, and extraction.

Additional Experiences:
Burlington Eye Associates - Burlington, MA
Medical Scribe – July 2014 – present

I am responsible for assisting the physician with documenting in the EMR and performing diagnostic eye procedures. There was a lot to learn including medical terminologies and common medications involved in ophthalmology. In addition, I had to learn how to use the EMR software, and be able to navigate the program in order to document patients’ medical record. I found it is crucial to have an open mind and a positive attitude in order to learn new things quickly.

BostonCares Volunteer – Boston, MA
Community Service volunteer – February 2014 – present

I volunteered at many different opportunities including tutoring adults to achieve their GED, serving meals to the homeless and veterans, building computers for schools in third world countries, packing food packages for the American Red Cross, and building beds for the homeless.

X-Cel Adult Education Tutor- Boston, MA
Math Tutor – February 2014- present

At X-Cel, I volunteered weekly to tutor adults who are working towards their GED. I find it very inspiring to see those who value their education and put in an effort into learning. Over time, I learned that the key is to have patience with the students, and be flexible by explaining concepts in different ways. Witnessing as well as having a part in their successes has been a very rewarding experience.

Brigham’s And Women’s Hospital – Boston, MA
Office Assistant – February 2014 – September 2014

I volunteered at the front office checking patients in, answering calls, and scheduling appointments. I was also responsible for filing charts, and preparing paperwork for patients. I communicated frequently with nurses,
physicians, and staff regarding patients’ charts and status. Working in the office, I was able to observe the various members of the healthcare team interact and work together to provide the best care for the patients.

Hospital Ambassador – October 2012 – May 2013

Assigned various task within the hospital. Delivered specimens and blood samples to different labs and departments. Admitted patients into their room, discharged patients, and transported patients to various places within the hospital. Frequently interacted directly with patients in a friendly manner. Worked closely with nurses and hospital staff in a proficient manner.

**Activities Information Maven** – September 2009-May 2013

*Student Activities Office, Boston University* – Boston, MA

Worked in an office as an information desk and selling tickets. Provide campus information to students and visitors. At nighttime, also had the role of the Escort Security Service. Maintained the student union by opening/locking doors that student groups needed to access. Also promoted various events on campus by social media and through selling tickets. Learned how to professionally interact with and serve customers/students. Worked extensively with social media to promote event and the office to get more reach and influence.


**Misc.:** BU Finance and Investment Club: September 2010 – May 2012

BU Ultimate Frisbee Club: September 2010- May 2011

Computer literacy: Microsoft (Word, Excel, PowerPoint, Access), HTML coding