# Somatic Mutations in Vascular Malformations

by

Daniel Aaron Snellings

Department of Molecular Genetics and Microbiology
Duke University

Date: _____

Approved:

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Douglas Marchuk, Supervisor

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Beth Sullivan

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Michael Hauser

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Timothy Reddy

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Craig Lowe

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Molecular Genetics and Microbiology in the Graduate School of Duke University
2021

# Abstract

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# Abstract

Write your abstract here. You should not include references or mathematical notation.

If you want to dedicate your thesis to anyone do so here

# Contents

# List of Tables

# List of Figures

# List of Abbreviations and Symbols

## Symbols

Put general notes about symbol usage in text here. Notice this text is double-spaced, as required.

| | |
|---|---|
| $\mathbb{X}$ | A blackboard bold $X$. Neat. |
| $\mathcal{X}$ | A caligraphic $X$. Neat. |
| $\mathfrak{X}$ | A fraktur $X$. Neat. |
| $\mathbf{X}$ | A boldface $X$. |
| $\mathsf{X}$ | A sans-serif $X$. Bad notation. |
| $\mathrm{X}$ | A roman $X$. |

## Abbreviations

Long lines in the `symbollist` environment are single spaced, like in the other front matter tables.

| | |
|---|---|
| AR | Aqua Regia, also known as hydrocloric acid plus a splash of nitric acid. |
| SHORT | Notice the change in alignment caused by the label width between this list and the one above. Also notice that this multiline description is properly spaced. |
| OMFGTXTMSG4ME | Abbreviations/Symbols in the item are limited to about a quarter of the textwidth, so don't pack too much in there. You'll bust the margins and it looks really bad. |

# Acknowledgements

Thank anyone you like here. It's good practice to thank every granting agency that's given you money since you've been ABD, any other school you visited during your research, and any professional society that's funded your travel.

# 1

# Introduction

# 2

# Two-Hit Mechanism of Hereditary Hemorrhagic Telangiectasia

## 2.1 Premise

## 2.2 Results

### *2.2.1 Telangiectasia Harmor a Somatic Mutation in ENG or ACVRL1*

### *2.2.2 Somatic and Germline Mutations are Biallelic*

### *2.2.3 Mutations are Consistent with Homozygous Loss of Function*

### *2.2.4 Telangiectasia from the Same Individual Harbor Unique Somatic Mutations*

## 2.3 Discussion

### *2.3.1 Evidence for a Genetic Two-Hit Mechanism*

### *2.3.2 Sensitivity for Detecting Somatic Mutations*

### *2.3.3 Necessary, but Not Sufficient*

### *2.3.4 Extent of Lesional Mosaicism*

### *2.3.5 Mutant Cell Metastasis*

### *2.3.6 Two-Hit Mechanism for SMAD4 & JP-HHT*

## 2.4 Methods

*Sample Collection*

*DNA and RNA Extraction*

*Targeted Sequencing*

*Mutation Detection*

*Establishing Phase*

*in vitro Splicing*

*Reverse-Transcription PCR*

# 3

# Mutant *GNAQ* Alleles Produce Distinct Disease Phenotypes

# 4

# *MAP3K3* Mutations Seed Cerebral Cavernous Malformations

# 5

# *PIK3CA* Mutations Fuel Cerebral Cavernous Malformation Growth

## 5.1 Premise

## 5.2 Results

### *5.2.1 PIK3CA Mutations Occur in Familial and Sporadic CCMs*

### *5.2.2 CCMs Harbor Multiple Somatic Mutations in Different Genes*

### *5.2.3 PIK3CA and CCM/MAP3K3 Mutations in the Same Cell*

### *5.2.4 Developmental Venous Anomalies Predispose to Malformation*

## 5.3 Discussion

### *5.3.1 Three-Hit Model of CCM Pathogenesis*

### *5.3.2 Similarities to the Genetic Mechanism of Cancer*

### *5.3.3 Role of Clonal Expansion in Mutagenesis*

### *5.3.4 Therapeutic Implications*

### *5.3.5 Distinct Properties of PIK3CA vs. CCM/MAP3K3 Mutations*

## 5.4 Methods

*CCM Collection*

*Brain AVM Collection*

*DNA Extraction*

*Droplet Digital PCR*

*SNaPshot*

*Sequencing*

*Sequence Analysis*

*Single-Nucleus DNA Sequencing*

*Statistics*

# 6

# Conclusion

6.1 Model for HHT Pathogenesis

6.2 Model for CCM Pathogenesis

6.3 Contribution of Somatic Mutations to Non-Cancer Diseases

# Appendix A

## Probability of Multiple Somatic Mutations

# Biography

Your biography is limited to one page and must contain

1. Full name
2. Date and place of birth
3. Every degree you've earned, including this one, and where you earned it from.

Mostly, that information is to narrow down which John Smith wrote that dissertation on the mating habits of sea cucumbers. Sexy!

You may also include

1. Any awards you've won related to your discipline since your undergraduate degree.
2. Any fellowships you've held
3. Anything you've published (papers, books, book chapters). Don't be afraid to cite it here, so that the full bibliographic record of your article appears in the bibliography!
4. Where your next job will be, if you know