

Somatic Mutations in Vascular Malformations

by

Daniel Aaron Snellings

Department of Molecular Genetics and Microbiology
Duke University

Date: _____

Approved:

Douglas Marchuk, Supervisor

Beth Sullivan

Michael Hauser

Timothy Reddy

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

Somatic Mutations in Vascular Malformations

by

Daniel Aaron Snellings

Department of Molecular Genetics and Microbiology
Duke University

Date: _____

Approved:

Douglas Marchuk, Supervisor

Beth Sullivan

Michael Hauser

Timothy Reddy

Craig Lowe

An abstract of a 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

Copyright © 2021 by Daniel Aaron Snellings
All rights reserved except the rights granted by the
Creative Commons Attribution-Noncommercial Licence

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

Abstract	iv
List of Tables	x
List of Figures	xi
List of Abbreviations and Symbols	xii
Acknowledgements	xiii
1 Introduction	1
1.1 Vascular Malformations	2
1.1.1 Common Genetic Mechanisms	2
1.2 Hereditary Hemorrhagic Telangiectasia	2
1.2.1 Genetics	2
1.2.2 Signaling of <i>ACVRL1</i> , <i>ENG</i> , and <i>SMAD4</i>	2
1.2.3 Relationship with Sporadic Arteriovenous Malformations	2
1.3 Sturge-Weber Syndrome	2
1.3.1 Mosaic Mutation of <i>GNAQ</i> p.R183Q	2
1.3.2 Function and Activity of <i>GNAQ</i>	2
1.3.3 Mutation of <i>GNAQ</i> in Other Diseases	2
1.4 Cerebral Cavernous Malformations	2
1.4.1 Genetics	2
1.4.2 Differences Between Familial and Sporadic Disease	2

1.4.3	Two-Hit Mechanism	2
1.4.4	Signaling of the CCM Complex and its Downstream Effectors	2
1.5	Infantile Hemangioma (??? Include Neg Data ???)	2
2	Two-Hit Mechanism of Hereditary Hemorrhagic Telangiectasia	3
2.1	Premise	4
2.2	Results	4
2.2.1	Telangiectasia Harbor a Somatic Mutation in <i>ENG</i> or <i>ACVRL1</i>	4
2.2.2	Somatic and Germline Mutations are Biallelic	4
2.2.3	Mutations are Consistent with Homozygous Loss of Function	4
2.2.4	Telangiectasia from the Same Individual Harbor Unique Somatic Mutations	4
2.3	Discussion	4
2.3.1	Evidence for a Genetic Two-Hit Mechanism	4
2.3.2	Sensitivity for Detecting Somatic Mutations	4
2.3.3	Necessary, but Not Sufficient	4
2.3.4	Extent of Lesional Mosaicism	4
2.3.5	Mutant Cell Metastasis	4
2.3.6	Two-Hit Mechanism for <i>SMAD4</i> & JP-HHT	4
2.4	Methods	4
3	Mutant <i>GNAQ</i> Alleles Produce Distinct Disease Phenotypes	5
3.1	Premise	5
3.2	Results	5
3.2.1	Port Wine Stain with Uncommon Mutation <i>GNAQ</i> p.Q209R	5
3.2.2	Structural Analysis of Common <i>GNAQ</i> Alleles	5
3.2.3	Functional Analysis of Common <i>GNAQ</i> Alleles (???Not sure it is appropriate to add this???)	5

3.2.4	Transcriptional Analysis of Common <i>GNAQ</i> Alleles	5
3.3	Discussion	5
3.3.1	Distinct Functions of <i>GNAQ</i> Alleles May Underlie Disease Specificity	5
3.3.2	Relationship Between <i>GNAQ</i> Activity and Mutation Timing .	5
3.3.3	Importance of Mutated Cell Type in Determining Disease Fate	5
3.4	Methods	5
4	<i>MAP3K3</i> Mutations Seed Cerebral Cavernous Malformations	6
4.1	Premise	6
4.2	Results	6
4.2.1	<i>MAP3K3</i> Somatic Mutations Only Occur in Sporadic CCM .	6
4.2.2	<i>MAP3K3</i> and CCM Gene Mutations are Mutually Exclusive .	6
4.2.3	Mutations in <i>KLF4</i> Do Not Contribute to CCM	6
4.2.4	(Whole-Exome Results) (??? Merge with above ???)	6
4.3	Discussion	6
4.3.1	CCM Loss of Function and <i>MAP3K3</i> Gain of Function are Functionally Equivalent	6
4.4	Methods	6
5	<i>PIK3CA</i> Mutations Fuel Cerebral Cavernous Malformation Growth	7
5.1	Premise	8
5.2	Results	8
5.2.1	<i>PIK3CA</i> Mutations Occur in Familial and Sporadic CCMs . .	8
5.2.2	CCMs Harbor Multiple Somatic Mutations in Different Genes	8
5.2.3	<i>PIK3CA</i> and CCM/ <i>MAP3K3</i> Mutations in the Same Cell . .	8
5.2.4	Developmental Venous Anomalies Predispose to Malformation	8
5.3	Discussion	8

5.3.1	Three-Hit Model of CCM Pathogenesis	8
5.3.2	Similarities to the Genetic Mechanism of Cancer	8
5.3.3	Role of Clonal Expansion in Mutagenesis	8
5.3.4	Therapeutic Implications	8
5.3.5	Distinct Properties of <i>PIK3CA</i> vs. CCM/ <i>MAP3K3</i> Mutations	8
5.3.6	DVA Predispose to CCM and Other PI3K-Related Diseases .	8
5.4	Methods	8
6	Developmental Venous Anomalies Predispose to Malformation	9
6.1	Premise	9
6.2	Results	9
6.3	Discussion	9
6.4	Methods	9
7	Conclusion	10
7.1	Model for HHT Pathogenesis	10
7.2	Model for CCM Pathogenesis	10
7.3	Contribution of Somatic Mutations to Non-Cancer Diseases	10
A	Probability of Multiple Somatic Mutations	11
	Biography	12
	Bibliography	12

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.
X	A boldface X .
X	A sans-serif X . Bad notation.
X	A roman X .

Abbreviations

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

AR	Aqua Regia, also known as hydrochloric 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

1.1 Vascular Malformations

1.1.1 Common Genetic Mechanisms

1.2 Hereditary Hemorrhagic Telangiectasia

1.2.1 Genetics

1.2.2 Signaling of ACVRL1, ENG, and SMAD4

1.2.3 Relationship with Sporadic Arteriovenous Malformations

1.3 Sturge-Weber Syndrome

1.3.1 Mosaic Mutation of GNAQ p.R183Q

1.3.2 Function and Activity of GNAQ

1.3.3 Mutation of GNAQ in Other Diseases

1.4 Cerebral Cavernous Malformations

1.4.1 Genetics

1.4.2 Differences Between Familial and Sporadic Disease

1.4.3 Two-Hit Mechanism

1.4.4 Signaling of the CCM Complex and its Downstream Effectors

1.5 Infantile Hemangioma (??? Include Neg Data ???)

Two-Hit Mechanism of Hereditary Hemorrhagic Telangiectasia

2.1 Premise

2.2 Results

2.2.1 Telangiectasia Harbor 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

Mutant *GNAQ* Alleles Produce Distinct Disease Phenotypes

3.1 Premise

3.2 Results

3.2.1 Port Wine Stain with Uncommon Mutation GNAQ p.Q209R

3.2.2 Structural Analysis of Common GNAQ Alleles

3.2.3 Functional Analysis of Common GNAQ Alleles (???Not sure it is appropriate to add this???)

3.2.4 Transcriptional Analysis of Common GNAQ Alleles

3.3 Discussion

3.3.1 Distinct Functions of GNAQ Alleles May Underlie Disease Specificity

3.3.2 Relationship Between GNAQ Activity and Mutation Timing

3.3.3 Importance of Mutated Cell Type in Determining Disease Fate

3.4 Methods

MAP3K3 Mutations Seed Cerebral Cavernous Malformations

4.1 Premise

4.2 Results

4.2.1 MAP3K3 Somatic Mutations Only Occur in Sporadic CCM

4.2.2 MAP3K3 and CCM Gene Mutations are Mutually Exclusive

4.2.3 Mutations in KLF4 Do Not Contribute to CCM

4.2.4 (Whole-Exome Results) (??? Merge with above ???)

4.3 Discussion

4.3.1 CCM Loss of Function and MAP3K3 Gain of Function are Functionally Equivalent

4.4 Methods

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

5.3.6 DVA Predispose to CCM and Other PI3K-Related Diseases

5.4 Methods

CCM Collection

Brain AVM Collection

DNA Extraction

Droplet Digital PCR

SNaPshot

Sequencing

Sequence Analysis

Single-Nucleus DNA Sequencing

Statistics

6

Developmental Venous Anomalies Predispose to Malformation

- 6.1 Premise
- 6.2 Results
- 6.3 Discussion
- 6.4 Methods

7

Conclusion

7.1 Model for HHT Pathogenesis

7.2 Model for CCM Pathogenesis

7.3 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