

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	xi
List of Figures	xii
List of Abbreviations and Symbols	xiii
Acknowledgements	xiv
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
4.3.2	Differing Constraints for Constitutional and Somatic Inheritance	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	Conclusions & Musings	9
6.1	HHT Pathogenesis	9
6.2	CCM Pathogenesis	9
6.2.1	Regrowth after Surgical Resection	9
6.2.2	CCM & Meningioma	9
6.3	Developmental Venous Anomalies as a Primer for Disease	10
6.3.1	Association with Sporadic CCM	10
6.3.2	Association with Other Diseases	10
6.3.3	Cowden Syndrome	10
6.3.4	Implications	10
6.4	Other Vascular Malformations	10
6.4.1	Classification of Vascular Malformations and Vascular Tumors	10
6.4.2	Sturge-Weber Syndrome and Somatic Mutations in <i>GNAQ</i>	10
6.4.3	The Curious Case of Infantile Hemangioma	10
6.5	The Molecular Basis of Genetic Dominance	13
6.5.1	Phenotypic Dominance \neq Genetic Dominance	13
6.5.2	Knudsons Fingerprint	13

6.5.3	The Diverse Functional Effects of Genetic Mutations	13
6.6	The Intersection of Somatic Mutagenesis and Evolution	13
6.6.1	The Creation of New Alleles	13
6.6.2	The Relationship Between Mutability and Fitness Landscape .	13
6.6.3	Recurring Mutations & Convergent Evolution	13
6.6.4	Clonal Evolution of Somatic Mutants	13
6.6.5	Cancer	13
6.7	Somatic Mutations	13
6.7.1	The Role of Somatic Mutations in Aging	13
6.7.2	Constitutional Intolerance & Somatic Permissiveness	13
6.7.3	Somatic Reversion of Pathogenic Mutations	13
6.7.4	What is the Consequence of RNA Mutations?	13
6.8	Innovation in the Sequencing Era	13
6.8.1	Detection of Somatic Mutations	13
6.8.2	Single Cell Sequencing	13
6.8.3	Utility of Rare Disease Research in Mechanistic Discovery . .	13
6.8.4	Data Democratization & Individual Privacy	13
6.8.5	Growing Importance of Informatics in Biology	13
A	Probability of Multiple Somatic Mutations	14
	Bibliography	15
	Biography	16

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 `sybollist` 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

4.3.2 Differing Constraints for Constitutional and Somatic Inheritance

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

Conclusions & Musings

6.1 HHT Pathogenesis

6.2 CCM Pathogenesis

6.2.1 Regrowth after Surgical Resection

6.2.2 CCM & Meningioma

Although the vascular lesions are the primary sequelae of familial CCM, many groups have noted an increased prevalence of meningioma in individuals with familial CCM—especially those with a mutation in *PDCD10* (Labauge et al., 2009; Riant et al., 2013; Garaci et al., 2015). In addition, we have previously been contacted by an individual whose child had a sporadic CCM that regrew into a meningioma. Unfortunately we were unable to acquire a tissue sample for genetic analysis, however this case along with the strong link between familial CCM and meningioma has fueled my interest in understanding the link between CCM and meningioma.

6.3 Developmental Venous Anomalies as a Primer for Disease

6.3.1 Association with Sporadic CCM

6.3.2 Association with Other Diseases

6.3.3 Cowden Syndrome

6.3.4 Implications

6.4 Other Vascular Malformations

6.4.1 Classification of Vascular Malformations and Vascular Tumors

6.4.2 Sturge-Weber Syndrome and Somatic Mutations in GNAQ

Happle's hypothesis

GNAQ in uveal melanoma and circumscribed choroidal hemangioma

Link between SWS, UM, and CCH?

6.4.3 The Curious Case of Infantile Hemangioma

Infantile hemangioma (IH) are one of the most common vascular malformations. They occur in children and are typically present at birth as a red spot flush with the surrounding skin. Soon after birth the hemangioma rapidly grows and becomes raised from the skin. They are generally benign and are typically left alone unless they cover the child's mouth, nose, or eyes. What makes IH so interesting compared to other types of vascular malformations is that they almost always completely regress within the first few years of the child's life. While other types of vascular malformations may spontaneously regress (telangiectasia and AVMs), none do so with the consistency of IH. This phenomenon has been of great interest, not for the purpose of developing therapeutics for IH (propranolol is an extremely effective treatment for IH) but for uncovering the mechanism of regression in the hopes that what we learn can be applied to regress other, more nefarious, vascular malformations.

GLUT1 in IH endothelium

Perhaps one of the most provocative discoveries into the mechanism of IH pathogenesis is the fact that endothelial cells from IHs highly express GLUT1 (North et al., 2000, 2001). GLUT1 is a glucose transporter that has remarkable specificity for the placental endothelium. This finding suggested that the IH may be comprised of cells that dislodged from the maternal placenta, then became hyper-proliferative in a post-fetal environment. If this hypothesis is correct, one would expect to find that the IH is a genetically chimeric growth between fetal and maternal cells. This hypothesis was put to the test using fluorescence *in situ* hybridization to assay the presence of XX cells in IH from a male infant with confirmation by sequencing microsatellites and SNPs that were divergent between mother and child. This analysis found no evidence for maternal-fetal chimerism in IH (Pittman et al., 2006). Despite this counter-evidence, the presence of GLUT1 in IH is strongly indicative of some link with the placenta though unfortunately this link currently remains elusive.

Efforts to find somatic mutations in IH

As it is quickly becoming clear that the vast majority of vascular malformations are the result of somatic mutations—many occurring in known oncogenes—I thought that somatic mutations may also underlie IH. To test this, I sequenced 61 IH lesions on an ‘oncopanel’ covering many genes that are highly mutated in cancers as well as several genes previously implicated in vascular malformations (*KRIT1*, *CCM2*, *PDCD10*, *ACVRL1*, *ENG*, *SMAD4*, etc.) Unfortunately after filtering putative variants, there were no variants with likely functional significance and that occurred in more than a single sample. I am aware of at least 1 other group that has attempted to identify somatic mutations in IH via whole-exome sequencing, however to date there are no known somatic mutations in IH. One important aspect of these studies that must be noted is that they are invariably focused on coding regions of

the genome. Non-coding variants are more than capable of causing disease however discovery-focused sequencing studies often ignore non-coding regions both because of the cost of sequencing the entire genome to a depth sufficient to detect somatic mutations, and the challenges associated with functional analysis of non-coding variants. Further studies may find that somatic mutations do cause IH, but they occur in a region of the genome that is missed by the majority of sequencing studies.

6.5 The Molecular Basis of Genetic Dominance

6.5.1 Phenotypic Dominance \neq Genetic Dominance

6.5.2 Knudsons Fingerprint

6.5.3 The Diverse Functional Effects of Genetic Mutations

6.6 The Intersection of Somatic Mutagenesis and Evolution

6.6.1 The Creation of New Alleles

6.6.2 The Relationship Between Mutability and Fitness Landscape

6.6.3 Recurring Mutations & Convergent Evolution

6.6.4 Clonal Evolution of Somatic Mutants

6.6.5 Cancer

6.7 Somatic Mutations

6.7.1 The Role of Somatic Mutations in Aging

6.7.2 Constitutional Intolerance & Somatic Permissiveness

6.7.3 Somatic Reversion of Pathogenic Mutations

6.7.4 What is the Consequence of RNA Mutations?

6.8 Innovation in the Sequencing Era

6.8.1 Detection of Somatic Mutations

6.8.2 Single Cell Sequencing

6.8.3 Utility of Rare Disease Research in Mechanistic Discovery

6.8.4 Data Democratization & Individual Privacy

6.8.5 Growing Importance of Informatics in Biology

Appendix A

Probability of Multiple Somatic Mutations

Bibliography

- Garaci, F., Marsili, L., Riant, F., Marziali, S., Cecillon, M., Pasquarelli, R., Sangiuolo, F., Floris, R., Novelli, G., Tournier-Lasserre, E., & Brancati, F. (2015). Cerebral cavernous malformations associated to meningioma: High penetrance in a novel family mutated in the *pdc10* gene. *Neuroradiol J*, *28*(3), 289–93.
URL <https://www.ncbi.nlm.nih.gov/pubmed/26246098>
- Labauge, P., Fontaine, B., Neau, J. P., Bergametti, F., Riant, F., Blecon, A., Marchelli, F., Arnoult, M., Lannuzel, A., Clanet, M., Olschwang, S., Denier, C., & Tournier-Lasserre, E. (2009). Multiple dural lesions mimicking meningiomas in patients with *ccm3/pdc10* mutations. *Neurology*, *72*(23), 2044–6.
URL <https://www.ncbi.nlm.nih.gov/pubmed/19506228>
- North, P. E., Waner, M., Mizeracki, A., & Mihm, J., M. C. (2000). Glut1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol*, *31*(1), 11–22.
URL <https://www.ncbi.nlm.nih.gov/pubmed/10665907>
- North, P. E., Waner, M., Mizeracki, A., Mrak, R. E., Nicholas, R., Kincannon, J., Suen, J. Y., & Mihm, J., M. C. (2001). A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch Dermatol*, *137*(5), 559–70.
URL <https://www.ncbi.nlm.nih.gov/pubmed/11346333>
- Pittman, K. M., Losken, H. W., Kleinman, M. E., Marcus, J. R., Blei, F., Gurtner, G. C., & Marchuk, D. A. (2006). No evidence for maternal-fetal microchimerism in infantile hemangioma: a molecular genetic investigation. *J Invest Dermatol*, *126*(11), 2533–8.
URL <https://www.ncbi.nlm.nih.gov/pubmed/16902414>
- Riant, F., Bergametti, F., Fournier, H. D., Chapon, F., Michalak-Provost, S., Cecillon, M., Lejeune, P., Hosseini, H., Choe, C., Orth, M., Bernreuther, C., Boulday, G., Denier, C., Labauge, P., & Tournier-Lasserre, E. (2013). *Ccm3* mutations are associated with early-onset cerebral hemorrhage and multiple meningiomas. *Mol Syndromol*, *4*(4), 165–72.
URL <https://www.ncbi.nlm.nih.gov/pubmed/23801932>

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