## Somatic Mutations in Vascular Malformations

by

## Daniel Aaron Snellings

Department of Molecular Genetics and Microbiology Duke University

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Douglas Marchuk, Supervisor
Beth Sullivan
Michael Hauser
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Timothy Reddy
Timothy Ready
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

## **Abstract**

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## Abstract

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

## Contents

A	bstra	$\operatorname{ct}$		iv	
Li	st of	Table	${f s}$	xi	
Li	List of Figures x				
Li	ist of	Abbre	eviations and Symbols	xiii	
A	cknov	wledge	ements	xiv	
1	Intr	oducti	ion	1	
	1.1	Vascu	lar Malformations	2	
		1.1.1	Common Genetic Mechanisms	2	
	1.2	1.2 Hereditary Hemorrhagic Telangiectasia			
		1.2.1	Genetics	2	
		1.2.2	Signaling of $ACVRL1$ , $ENG$ , and $SMAD4$	2	
		1.2.3	Relationship with Sporadic Arteriovenous Malformations	2	
1.3 Sturge-Weber Syndrome			e-Weber Syndrome	2	
		1.3.1	Mosaic Mutation of $GNAQ$ p.R183Q	2	
		1.3.2	Function and Activity of GNAQ	2	
		1.3.3	Mutation of $\mathit{GNAQ}$ in Other Diseases	2	
	1.4	Cereb	ral 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	Infant	ile Hemangioma (??? Include Neg Data ???)	2	
2	Two	o-Hit I	Mechanism of Hereditary Hemorrhagic Telangiectasia	3	
	2.1	1 Premise		4	
	2.2	SS	4		
		2.2.1	Telangiectasia Harmor a Somatic Mutation in $\it ENG$ or $\it ACVRL1$	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			
		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 $SMAD4$ & JP-HHT	4	
	2.4	Metho	ods	4	
3	Mu	tant G	ENAQ Alleles Produce Distinct Disease Phenotypes	5	
	3.1	Premise		5	
	3.2	Result	SS	5	
		3.2.1	Port Wine Stain with Uncommon Mutation $\mathit{GNAQ}$ p.Q209R .	5	
		3.2.2	Structural Analysis of Common $\mathit{GNAQ}$ 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 $GNAQ$ Alleles	5
	3.3	Discus	sion	5
		3.3.1	Distinct Functions of $GNAQ$ Alleles May Underlie Disease Specificity	5
		3.3.2	Relationship Between $\mathit{GNAQ}$ Activity and Mutation Timing .	5
		3.3.3	Importance of Mutated Cell Type in Determining Disease Fate	5
	3.4	Metho	$\mathrm{d}\mathrm{s}$	5
4	MA	P3K3	Mutations Seed Cerebral Cavernous Malformations	6
	4.1	Premi	se	6
	4.2	Result	s	6
		4.2.1	MAP3K3 Somatic Mutations Only Occur in Sporadic CCM $$ .	6
		4.2.2	$\mathit{MAP3K3}$ and CCM Gene Mutations are Mutually Exclusive .	6
		4.2.3	Mutations in $\mathit{KLF4}$ Do Not Contribute to CCM	6
		4.2.4	(Whole-Exome Results) (??? Merge with above ???)	6
4.3 Discussion			sion	6
		4.3.1	CCM Loss of Function and $MAP3K3$ Gain of Function are Functionally Equivalent	6
		4.3.2	Differing Constraints for Constitutional and Somatic Inheritance	6
	4.4	Metho	ds	6
5	PII	IK3CA Mutations Fuel Cerebral Cavernous Malformation Growth		
	5.1	.1 Premise		8
	5.2	2 Results		
		5.2.1	$\it PIK3CA$ Mutations Occur in Familial and Sporadic CCMs $$ .	8
		5.2.2	CCMs Harbor Multiple Somatic Mutations in Different Genes	8
		5.2.3	$PIK3CA$ and $\mathrm{CCM}/MAP3K3$ 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 $PIK3CA$ vs. $CCM/MAP3K3$ Mutations	8
		5.3.6	DVA Predispose to CCM and Other PI3K-Related Diseases $$ .	8
	5.4	Metho	ods	8
6	Con	clusio	ns & Musings	9
	6.1	ннт	Pathogenesis	10
	6.2	CCM	Pathogenesis	10
	6.3	6.3 Developmental Venous Anomalies as a Primer for Disease		
		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	Unique Properties of Somatic Mutations in $\mathit{GNAQ}$	10
		6.4.3	The Curious Case of Infantile Hemangioma	10
	6.5	6.5 Genetic Mechanisms of Mendelian Disease		
		6.5.1	Phenotypic Dominance $\neq$ Genetic Dominance	10
		6.5.2	Knudsons Fingerprint	10
		6.5.3	The Diverse Functional Effects of Genetic Mutations	10
	6.6	The In	ntersection of Somatic Mutagenesis and Evolution	10

		6.6.1	The Creation of New Alleles	10
		6.6.2	The Relationship Between Mutability and Fitness Landscape .	10
		6.6.3	Recurring Mutations & Convergent Evolution	10
		6.6.4	Clonal Evolution of Somatic Mutants	10
		6.6.5	Cancer	10
6	5.7	Somati	ic Mutations	10
		6.7.1	The Role of Somatic Mutations in Aging	10
		6.7.2	Constitutional Intolerance & Somatic Permissiveness $\ \ldots \ \ldots$	10
		6.7.3	Somatic Reversion of Pathogenic Mutations	10
		6.7.4	What is the Consequence of RNA Mutations?	10
6	5.8	Innova	tion in the Sequencing Era	10
		6.8.1	Detection of Somatic Mutations	10
		6.8.2	Single Cell Sequencing	10
		6.8.3	Data Democratization & Individual Privacy	10
		6.8.4	Growing Importance of Informatics in Biology	10
A F	Prol	babilit	y of Multiple Somatic Mutations	11
Biog	Biography			12
Bibl	Bibliography			

# 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.
- X A sans-serif X. Bad notation.
- 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.

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 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

# 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

# $PIK3CA \ \, {\rm Mutations} \ \, {\rm Fuel} \ \, {\rm Cerebral} \ \, {\rm Cavernous} \\ {\rm Malformation} \ \, {\rm Growth}$

#### 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** 

Conclusions & Musings

- 6.1 HHT Pathogenesis
- 6.2 CCM Pathogenesis
- 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 Unique Properties of Somatic Mutations in GNAQ
- 6.4.3 The Curious Case of Infantile Hemangioma
- 6.5 Genetic Mechanisms of Mendelian Disease
- 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

# 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