UC Irvine
UC Irvine Previously Published Works

Title
The somatic GNAQ mutation (R183Q) is primarily located within the blood vessels of port wine stains

Permalink
https://escholarship.org/uc/item/13r8167c

Journal
Journal of the American Academy of Dermatology, 74(2)

ISSN
0190-9622

Authors
Tan, Wenbin
Nadora, Dawnica Mercado
Gao, Lin
et al.

Publication Date
2016-02-01

DOI
10.1016/j.jaad.2015.09.063

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

J Am Acad Dermatol. Author manuscript; available in PMC 2017 February 01.

Published in final edited form as:

J Am Acad Dermatol. 2016 February ; 74(2): 380–383. doi:10.1016/j.jaad.2015.09.063.

The Somatic GNAQ Mutation (R183Q) is Located within the Blood Vessels of Port Wine Stains

Wenbin Tan, Ph.D.[1,*], **Dawnica Mercado Nadora, B.S.**[1], **Lin Gao, M.D., Ph.D.**[1,4], **Gang Wang, M.D., Ph.D.**[4], **Martin C. Mihm Jr., M.D.**[3], and **J. Stuart Nelson, M.D., Ph.D.**[1,2]

[1]Department of Surgery, Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, California 92617, USA

[2]Department of Biomedical Engineering, University of California, Irvine, Irvine, California 92617, USA

[3]Department of Dermatology, Brigham and Women's Hospital, Harvard Institute of Medicine, Boston, Massachusetts 02115, USA

[4]Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, 710032, China

To the Editor

Port wine stain (PWS) is a congenital vascular malformation of human skin involving the superficial vascular plexus, occurring in an estimated 3–5 children per 1,000 live births.[1] A sporadic somatic guanine nucleotide-binding protein, G alpha subunit q (GNAQ) mutation (R183Q) has been identified in PWS lesions.[2, 3] However, the cell-type specific distributions of the GNAQ mutation (R183Q) in PWS lesions have yet to be determined.

The study was approved by the Institutional Review Board at the University of California, Irvine. The clinical histories of PWS biopsy samples were described in a previous study.[4] In order to identify which skin structure enriches the GNAQ (R183Q), we performed laser capture microscopy (LCM) to collect blood vessels and three other structures within PWS lesional skin, namely, epidermis, hair follicles/glands and connective tissues, on formalin-fixed paraffin embedded (FFPE) sections. An outline of LCM and DNA library construction is illustrated in Figure 1.

Correspondence: Wenbin Tan, Ph.D, Department of Surgery, Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, California 92617, USA. wenbint@uci.edu Phone: 949-824-3754 Fax: 949-824-6969.

Financial Disclosure: None declared.

DISCLAIMER Any views expressed here represent personal opinion and do not necessarily reflect those of the U.S. Department of Health and Human Services or the United States federal government.

PRIOR PUBLICATION: None of the material in this manuscript has been published or is under consideration for publication elsewhere, including the Internet.

IRB status statement: The study was approved by the Institutional Review Board at the University of California, Irvine.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

We set the mutation frequency exceeding 1% as a mutation positive sample as described by Shirley et al.[2] We identified the GNAQ (R183Q) in 8/10 PWS subjects (80%) through the next generation sequencing (NGS) method (Table 1). The mutation was primarily located within the blood vessels in PWS lesions (6/10 subjects) with frequencies ranging from 3.16 to 12.37%. Two of those subjects also showed the same mutation in the connective tissues with frequencies of 22.17 and 6.43%, respectively (Table 1). There were no mutations found in the epidermis or hair follicle/glands in those 6 subjects PWS lesions (Table 1).

Two other subjects showed the mutation located in connective tissues and/or hair follicle/glands with frequencies ranging from 2.67 to 6.62%, but not in the blood vessels or the epidermis of PWS lesions (Table 1). The remaining two subjects were found to be negative for this mutation in all four dissected structures within PWS lesions (Table 1). There were no mutations found in normal control skin adjacent to PWS sites or normal dermal blood vessels from a healthy subject (Table 1).

GNAQ (R183Q) induces minimal activation of MAPK in a cell culture system.[2] Whether it can activate the same signaling pathway in PWS lesions remains incompletely understood. We recently found the c-Jun N-terminal kinases (JNK) and extracellular signal regulated kinases (ERK) were consecutively activated in both infantile and adult PWS blood vessels.[4] Here we further identified that the GNAQ (R183Q) was primarily located within blood vessels in PWS lesions from 60% of our subjects, suggesting a causative correlation between GNAQ (R183Q) and activation of JNK and ERK in this subset of subjects under study. The reason for GNAQ (R183Q) occurrence in connective tissues and/or hair follicle/glands, but not blood vessels, in some subjects is unknown and requires further investigation. The fact that GNAQ (R183Q) occurrence in both blood vessels and connective tissues suggests that pluripotent cells with the GNAQ (R183Q) may give rise to multilineages in PWS. We conclude that enrichment of GNAQ (R183Q) in PWS blood vessels may induce consecutive activation of JNK and ERK, thus contributing to the pathogenesis of PWS.

Acknowledgments

Funding/Support: This study was supported in part by NIH AR063766 to WT, AR47551 and AR59244 to JSN, and the American Society for Laser Medicine and Surgery research grants F03.12 and F01.13 to WT.

We greatly appreciate the assistance of the Sue & Bill Gross Stem Cell Research Center and the Genomics High-Throughout Facility at the University of California, Irvine, on the laser capture and next–generation sequencing experiments. Institutional support was provided by the Arnold and Mabel Beckman Foundation and the David and Lucile Packard Foundation.

References

1. Mulligan PR, Prajapati HJ, Martin LG, et al. Vascular anomalies: classification, imaging characteristics and implications for interventional radiology treatment approaches. Br J Radiol. 2014; 87:20130392. [PubMed: 24588666]
2. Shirley MD, Tang H, Gallione CJ, et al. Sturge-Weber Syndrome and Port-Wine Stains Caused by Somatic Mutation in GNAQ. N Engl J Med. 2013; 368:1971–9. [PubMed: 23656586]
3. Lian CG, Sholl LM, Zakka LR, et al. Novel Genetic Mutations in a Sporadic Port-Wine Stain. JAMA Dermatol. 2014; 150:1336–40. [PubMed: 25188413]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. Tan W, Chernova M, Gao L, et al. Sustained activation of c-Jun N-terminal and extracellular signal-regulated kinases in port-wine stain blood vessels. J Am Acad Dermatol. 2014; 71:964–68. [PubMed: 25135651]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

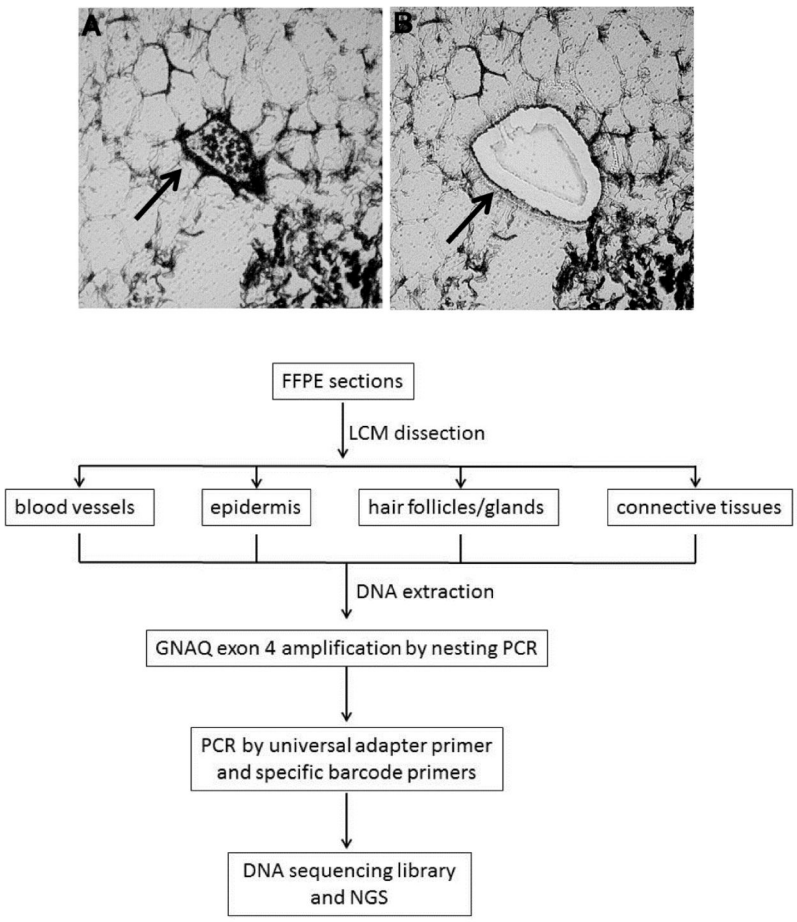

Figure 1. The outline of laser capture microscopy (LCM), DNA library construction and next generation sequencing (NGS)

An example of a PWS blood vessel indicated by the arrow in a FFPE section (A) was dissected by a laser beam in (B) under LCM. Four different structures within PWS lesional or normal control skin, namely, blood vessels, epidermis, hair follicles/glands and adjacent connective tissues were collected under LCM from FFPE sections, followed by DNA extraction. GNAQ exon 4 fragment was amplified by a nesting PCR. The PCR products then were purified from the gel and amplified by a universal adapter primer and a specific barcode primer. During PCR, each sample was designated by a specific barcode with a total of 48 barcodes being used. PCR products from each sample were purified, quantified and pooled together in equal quantity to make a sequencing library. NGS was performed on an Illumina HiSeq 2500 (Illmuina, San Diego, CA).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

The somatic mutations of GNAQ (R183Q) in different dissected structures of PWS lesional skin

Subject number	Age/Biopsy location		Structures	Mutant allele reads/wild type reads	Mutant allele frequency (%)	Negative or Positive
1	0	S#	BV	240/188949	0.127	
			EP	366/205108	0.178	
			HG	12128/18369	6.193	Positive
			CT	307/203632	0.150	
		C#	BV	216/212836	0.101	
2	0	Ex#	BV	24966/176781	12.375	Positive
			EP	312/186507	0.176	
			HG	438/197361	0.221	
			CT	45467/159605	22.171	Positive
		C#	BV	237/134826	0.176	
			EP	384/204481	0.187	
			HG	336/205254	0.163	
			CT	359/200501	0.179	
3	13	Ex#	BV	6735/206598	3.162	Positive
			EP	274/198700	0.138	
			HG	254/212979	0.119	
			CT	273/191881	0.142	
4	16	S#	BV	466/182249	0.255	
			EP	1804/178880	0.998	
			HG	881/176383	0.497	
5	27	S#	BV	22196/183535	10.789	Positive
			EP	549/206683	0.265	
			HG	399/205428	0.194	
			CT	454/206598	0.219	
6	38	F#	BV	6434/192558	3.233	Positive
7	39	F#	BV	12035/181885	6.206	Positive
			EP	452/203603	0.221	

J Am Acad Dermatol. Author manuscript; available in PMC 2017 February 01.

Subject number	Age/Biopsy location		Structures	Mutant allele reads/wild type reads	Mutant allele frequency (%)	Negative or Positive
			HG	457/202402	0.225	
			CT	12476/181486	6.432	Positive
8	41	F#	BV	270/204200	0.132	
			EP	228/210742	0.108	
			HG	279/209168	0.130	
			CT	270/206942	0.130	
		C#	BV	413/184457	0.223	
			EP	299/197275	0.151	
			CT	315/190720	0.165	
9	51	F#	BV	22889/178537	11.363	Positive
			EP	325/202807	0.160	
			HG	327/200564	0.163	
			CT	274/204250	0.134	
10	56	F#	BV	270/221245	0.122	
			EP	297/186705	0.159	
			HG	329/140943	0.233	
			CT	12801/180509	6.622	Positive
		N#	BV	n.a.	n.a.	
			HG	5559/202651	2.670	Positive
			CT	342/207006	0.165	
normal control	76	Ex#	BV	256/209472	0.122	

S#:scalp; Ex#:extremity; F#: facial; C#: the adjacent normal skin control from the same subject; N#: Neck; n.a., data not available.

BV: blood vessel; EP: epidermis; HG: hair follicle/gland; CT: connective tissue

Author Manuscript Author Manuscript Author Manuscript Author Manuscript