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Supplemental Data

Somatic Activating Mutations in *GNAQ* and *GNA11* Are Associated with Congenital Hemangioma

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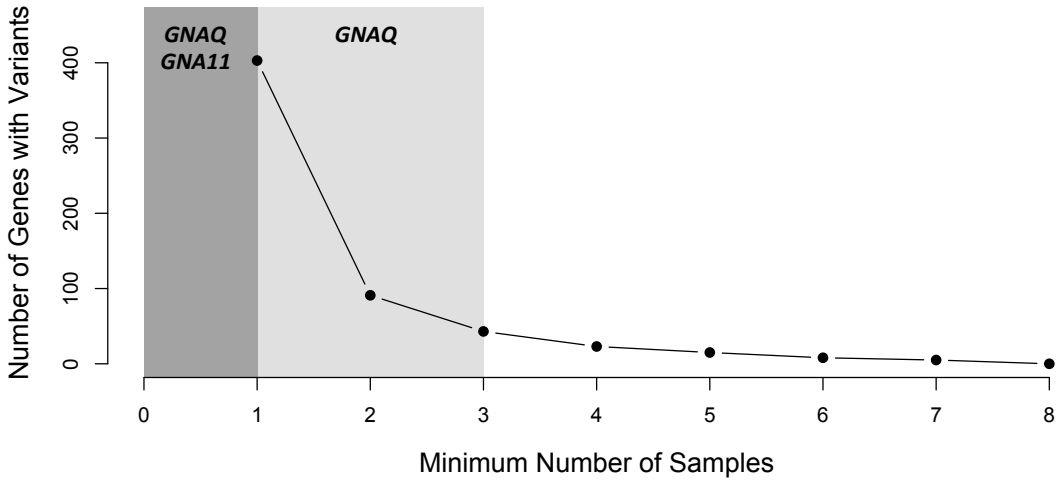

Figure S1: The number of genes with variants as a function of the number of samples in which they were detected. Four hundred and three genes with at least one variant were detected in at least one sample. Using our initial filtering criteria, *GNAQ* variants were detected in 3 samples, whereas a *GNA11* variant was detected in only 1 sample.

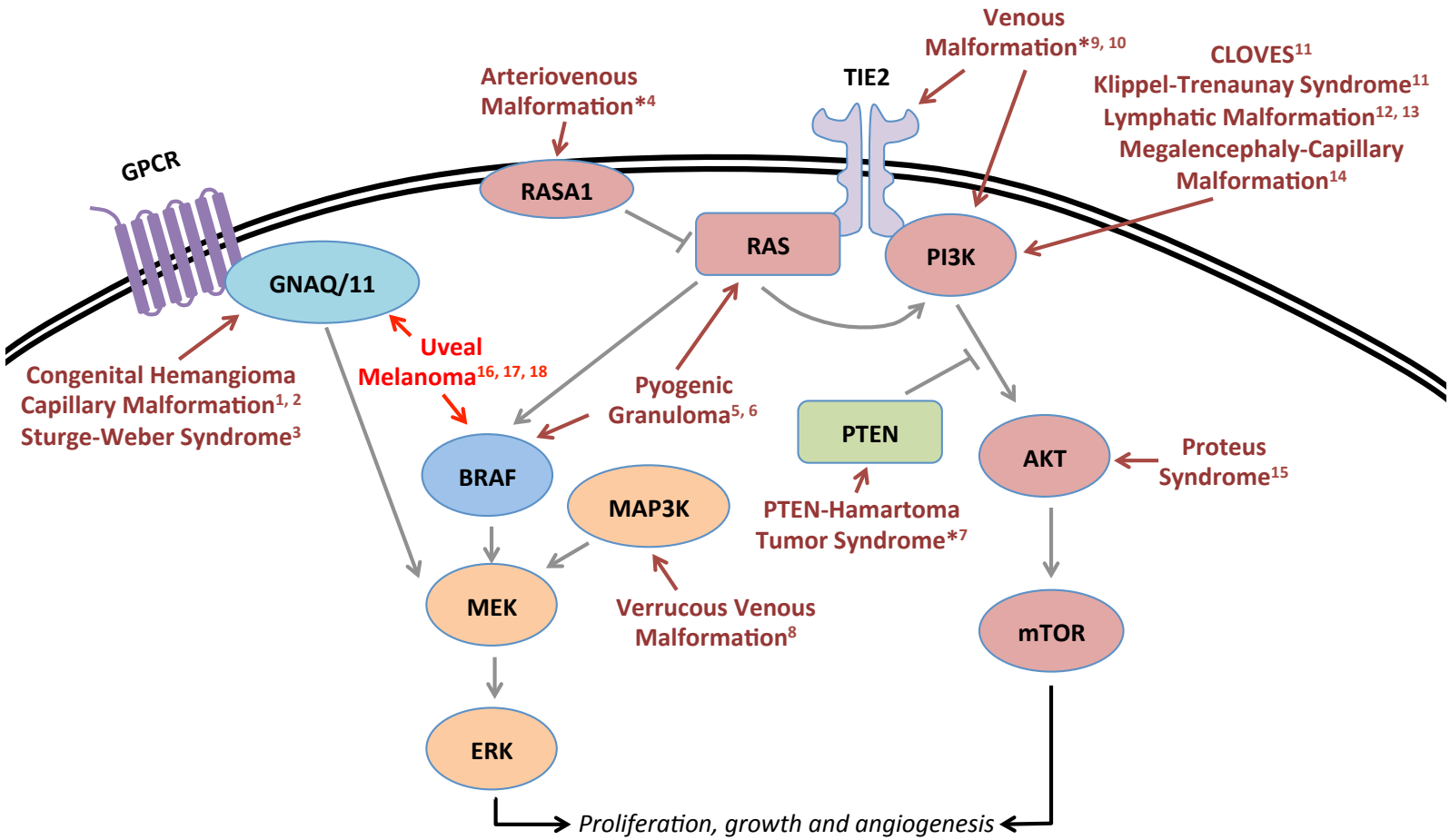

Figure S2: Somatic mutations in genes that are associated with congenital vascular tumors and malformations in humans. Mutations in genes encoding several different protein components of MAP kinase and mTOR signaling pathways have been identified in affected tissue from individuals with a vascular tumor or malformation. Relative locations and connections between different components in the signaling pathways are indicated by grey lines (arrowheads indicate a component that normally increases signaling and bars indicate a component that normally inhibits signaling). Colored text indicates the specific disorders and colored arrows point to pathway component(s) that can be responsible for causing the disorder [1-18]. A condition for which a germline mutation may also be causal is indicated with an asterisk.

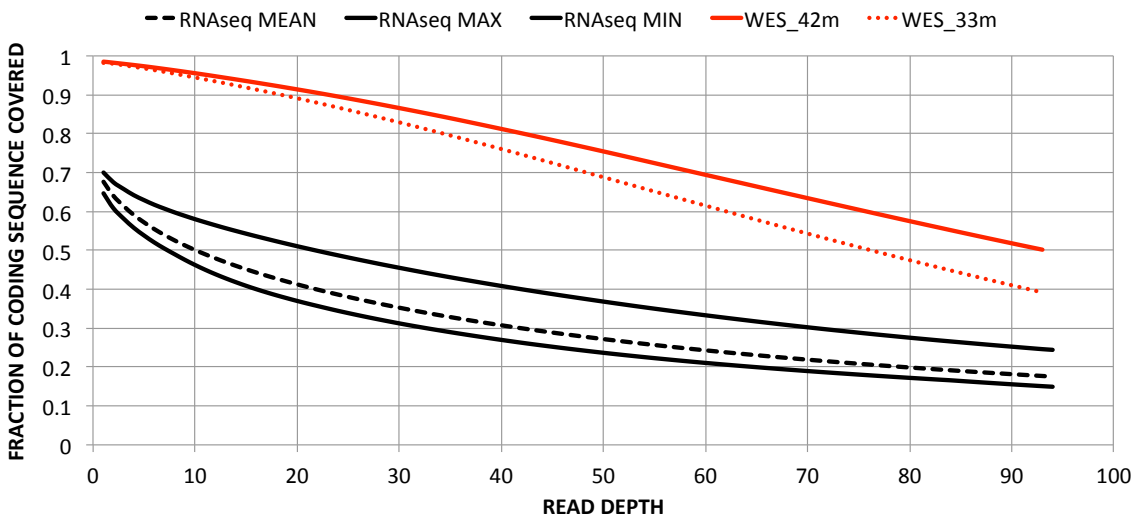

Figure S3: Fraction of the protein coding sequence covered by RNA-seq and representative whole exome data as a function of read depth. Whole exome datasets (derived from non-hemangioma tissue, unpublished) are generated with 33-42 million reads, making them comparable to the RNA-seq datasets generated with 32-47 million reads per library.

Gene Symbol	Chr	Pos	Ref/Var	Evaluation Notes
ATN1	12	7045882	G/T	Likely misalignment: Variants are present in a region with highly repeated sequence. The reads can map to the same region with a 6bp in-frame deletion and without mismatches.
		7045885	A/C	
		7045888	G/T	
		7045891	A/C	
		7045894	G/C	
		7045924	G/T	
B3GNT2	2	62449455	G/A	Likely sequencing error: The variant is in the middle of a repeated A sequence. Several other reads indicate single base-pair deletions/insertions (A).
GNAQ	9	80409487	T/G	No visible evidence of mismapping or sequencing errors. The altered codon is highly conserved.
		80409488	T/A	
IL13RA1	X	117892031	G/A	Likely sequencing error: The variant is in the middle of a repeated A sequence. Several other reads indicate single base-pair insertions (A).
NBPF11	1	146052732	G/C	Possible misalignment: The reads could be mapped to other positions with better scores.
		146057326	A/T	
		146057343	C/A	
		147579272	A/G	
PLEKHO1	1	150131221	G/A	Likely sequencing error: The variant is at the end of a repeated A sequence. Several other reads indicate single base-pair insertions (A).
SMC4	3	160131304	G/A	Likely sequencing error: The variant is in the middle of a repeated A sequence. Several other reads indicate single base-pair deletions/insertions (A).
		160134135	G/A	
SSC5D	19	56029182	A/C	Potential sequencing/mapping error: The variants are present in repetitive GC-rich regions at low level, and are accompanied by several other mismatches.
		56029484	C/G	
		56029524	A/C	
		56030202	C/A	
TBX3	12	115115392	C/T	Likely sequencing error: The variant is at the end of a repeated T sequence. Several other reads indicate single base-pair insertions (T).
TCF7L2	10	114925316	G/A	Likely sequencing error: The variant is at the end of a repeated A sequence. Several other reads indicate single base-pair insertions (A).
TCHP	12	110344434	G/A	Likely sequencing error: The variant is in the middle of a repeated A sequence. Several other reads indicate single base-pair insertions (A).
POLR2J2/UPK3BL	7	102280786	T/G	Possible misalignment: The reads could be mapped to other positions with similar scores.

Table S1: The variants identified in 12 genes by filtering RNA-seq data from 8 participants.

Primer Sequences

GNAQ fwd:5'-TTCCCTAAGTTTGTAAGTAGTGCT-3'; rev:5'-TCCATTGCCTGTCTAAAGAACAC-3'

GNA11 fwd: 5'-CAGCCGATGTCAGTCTGGT-3'; rev: 5'-GGCGACGAGAAACATGATGGA-3'

Probe Sequences

GNAQ Reference: 5'-/5HEX/CTTCTCTCTGACCTTTGGCCCCCTA/3IABkFQ/-3';

GNA11 Reference: 5'-/5HEX/CGCTCCGACCGCTGGCC/3IABkFQ/-3'

GNAQ.c626A>T: 5'-/56-FAM/TCTCTCTGACCTTAGGCCCCCTAC/3IABkFQ/-3'

GNAQ.c626A>C: 5'-/56-FAM/TCTCTCTGACCTTGGGCCCCCTAC/3IABkFQ/-3'

GNA11.c626A>T: 5'-/56-FAM/ACCGCAGGCCCCCCACA/3IABkFQ/-3'

Table S2: Sequences of primers and probes used in the ddPCR assays.

Probe ID	MIP sequence
GNAQ_1	GTCACTGTCTGGGTTCAGGTCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGGCGGGTGTCTAGGAGG
GNAQ_2	CGGCCAAGAGACAAGAGGGACACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGCAAAGACAAAGCGGATA
GNAQ_3	GTTCTAAAGAGAGCCTTGCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGCTGTGGTATGAGTGCTGACT
GNAQ_4	ACCCCTGGTTCCAGAACTCCTCGGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGATGGGTTGGACTTAT
GNAQ_5	GCCCCCTACATCGACCATTCTGCACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTCCATTGCCTGTCTAA
GNAQ_6	TGACGATGATCATCCAAGTCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTTTCTTCTCTCTGACCTTTG
GNAQ_7	CAGGGTCAGCTACGCGGTCCAAGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNAGGCATAAAAGCTGGG
GNAQ_8	GCTTAGAGTTCGAGTCCCCACCACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTTTTTGTCCTTCCCTT
GNAQ_9	CAGGTGGCCCTATGGATTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGTTGATGTGGAGAAGGTGTCTG
GNAQ_10	CCTGGAATCCAGGAATGCTATGATCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTGGAAAGTAATAGGGT
GNAQ_11	GCGACTCTTCATTGGAGCAGTCAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGGTCAGGATACTCTG
GNAQ_12	CAGAACATCTTCACGGCCATGCAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGTGGTCTGATGAGCTG
GNAQ_13	GTGAGTACCGTCCGGGGCCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTGGAAGAATGACTCTGGAGTC
GNAQ_14	GATCGAGCGGCAGCTCCGCAGGGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGAGGGTGTGTGTGCG
GNA11_1	GCGAGCTCAAGCTGCTGCTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNAGGCGGCCGCGTCGGCCGGGG
GNA11_2	ACTCCAGAGTCATCGTCCCGGCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGGGGCCGCACTCACCGA
GNA11_3	GCACGTGAGGGCGGGCGGCAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTGCATGGCGGTGAAGATGT
GNA11_4	GCTTGGTGGTGAGCATGGTGGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNAAGCTCGTCTACCAGAACA
GNA11_5	CGTGGATGATGCGCATCTGCTTGACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNCTCACCTTGTTCTGCT
GNA11_6	CCCTGTGGGAGGACCCGGGCATCCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTTCTGGTGGATGTGTGG
GNA11_7	TAAGTGCGGCCGCACCGCTGGCGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGGAGGTGGACGTGGAG
GNA11_8	GCTGCAACACAGCGGTGGGTGCTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGGCGGTACCGGAAGAT
GNA11_9	CGCCTGAGTCCAGGAGTTTGAGACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTTCGACCTGGAGAACA
GNA11_10	GACGGAAAGCCACCAGGAGGGGGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTGCTGGGTGGGCAGGT
GNA11_11	GTGGAGTCGGACAACGAGGTGGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNTGAGTCCTGGCGCTGTGT
GNA11_12	TGCCCTGAGCAGGGGCAGCGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGATGGTGGATGTGGGGGG
GNA11_13	GCTGATATGGGAGAGGGGCTCATACTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNCTTCCTCAACAAGAAG
GNA11_14	GCGAAGGCAGAGGGAATCAGAGGGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGTGCGAGTACAGGAT
GNA11_15	GGAGGGCAGAGGGTGAGGCTGTCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNAACACGAAGCGGATGTTC
GNA11_16	GCAGCTCAACCTCAAGGAGTACCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGGCGGCGCGGGAGTTCAT
GNA11_17	CGGTGGCACACGTGAAGTGTGAGCTTCAGCTTCCCGATATCCGACGGTAGTGTNNNNNNNGTGGAAGGAAGGTCCTG

Table S3: Sequences of probes used in the MIP-seq assays.

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