

Deep amplicon sequencing reveals *GNAQ* 548G→A as the causal somatic mutation in Sturge-Weber syndrome and common port-wine stains.

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Overview

- Pathology of the Sturge-Weber syndrome
- Somatic vs. germline variant detection
- Discovery from whole genome sequencing
- Validation by deep amplicon sequencing
- Biology of *GNAQ* mutations

Pathology of the Sturge-Weber syndrome

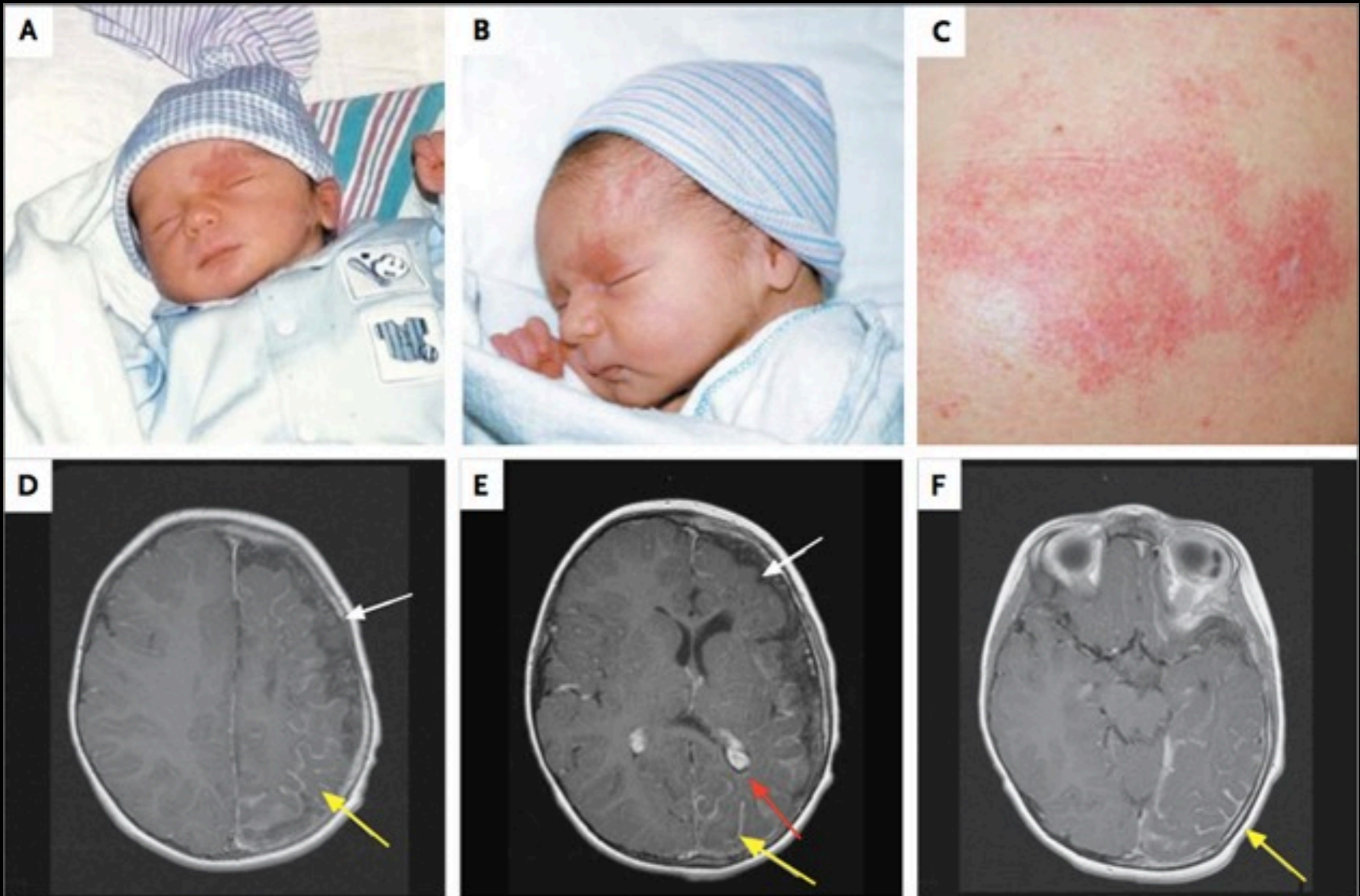
- Port-wine stain (PWS) affecting the face
- Abnormal capillary blood vessels in the brain
- Results in:
 - seizures
 - stroke
 - glaucoma
 - intellectual disability



Diagnosis in the general population

- SWS: approximately 1 in 20-50,000 live births
- 6-26% probability for children with a facial PWS
- PWS: approximately 3 in 1000 live births





Shirley, M. D. et al. (2013). Sturge–Weber Syndrome and Port-Wine Stains Caused by Somatic Mutation in *GNAQ*. *New England Journal of Medicine*.

Hypothesis about SWS etiology?

- Always occurs sporadically
- Lesions are distributed in a mosaic pattern
- Variable extent of involved tissue
- Localized - phenotype does not spread
- Sex ratio is 1:1

Happle, R. (1987). Journal of the American Academy of Dermatology

Hypothesis about SWS etiology?

- Rudolf Happle: SWS is caused by a somatic activating mutation escaping lethality during development¹
- Happle was proven correct for McCune-Albright² (*GNAS*) and Proteus³ (*AKT*) mutations

1. Happle, R. (1987). *Journal of the American Academy of Dermatology*

2. Weinstein, L. S. et al. (1991). *New England Journal of Medicine*.

3. Lindhurst, M. et al. (2011). *New England Journal of Medicine*.

Somatic vs. germ-line variant detection

Germ-line variant detection

- 3 states: A|A (0%), A|B (50%), B|B (100%)

- Heterozygous 30X coverage example:

AAAAAAAAAAAAAAAAABBBBBBBBBBBBBBBBBB

- Signal (B) is greater than noise (N)

AAAAAAAAANAAAAAAAAABBBBBBBBBBBBBBBNBB

= 46.7% A, 46.7% B, 6.6% N

Somatic variant detection

- States are not discrete
- Mixed abnormal/normal sample contamination
- 30X average genome sequence:

AABB

- Signal (**B**) is *small*, noise (**N**) is *overwhelming*

AAAAANAAAAAAAAAANAANAAAAAAAAAAAAAAAABB

= 85.7% A, 7.1% B, 7.1% N

Low frequency variants will be difficult to detect.

Discovery from whole genome sequencing (WGS)

Discovery from whole genome sequencing

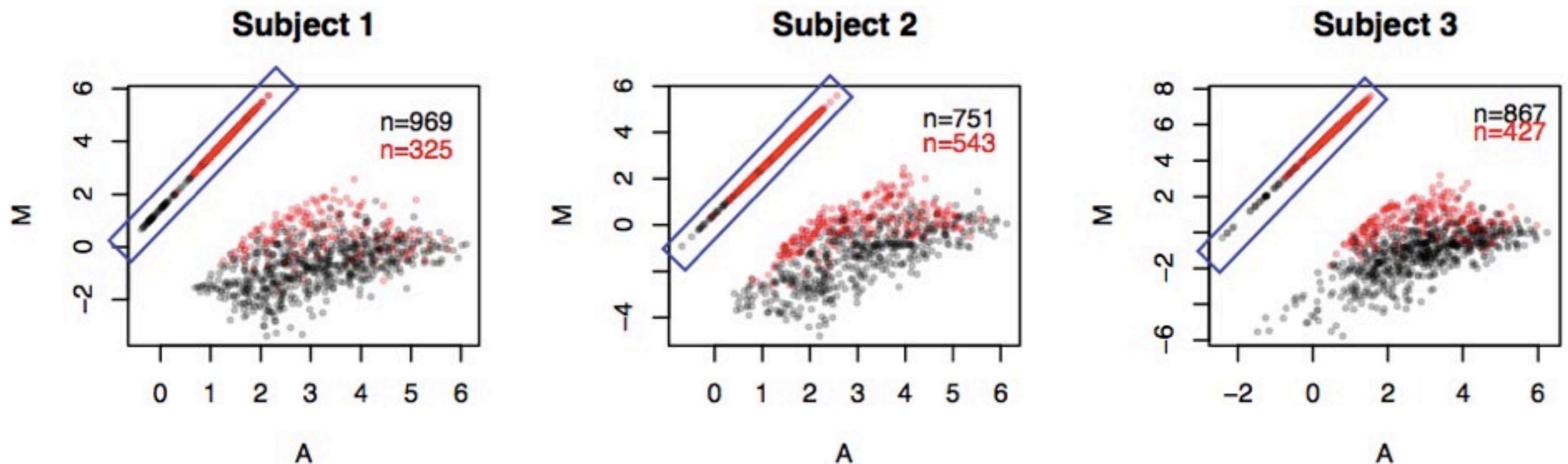
- Achieved 33-51X average coverage in 6 paired samples from 3 subjects
- Matched normal / affected tissue samples
- Strelka somatic genotyper

Subject	Somatic SNVs in abnormal	Normal	Affected
1	325	Skin	PWS
2	543	Brain	Brain
3	427	Skin	PWS

No shared variants

Discovery from whole genome sequencing

- Concerned about missing low frequency somatic variants
- Look at all call sites for any alternate alleles in affected (absent normal)



M = sum of log transformed allele frequencies

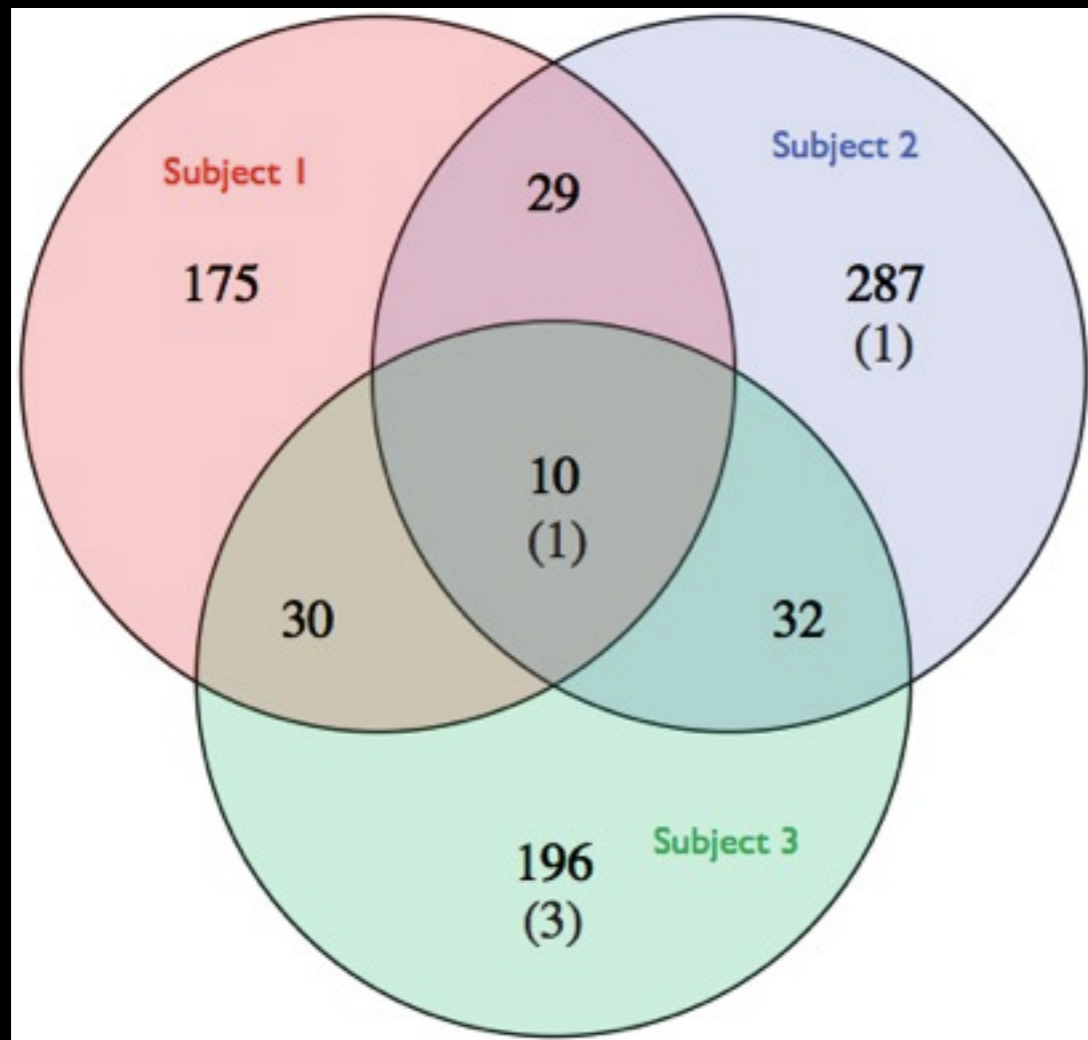
A = difference of log transformed allele frequencies

Red = variants called in subject

Black = variants called in other subjects

Blue box: variants having a mutant allele only in affected sample

Discovery from whole genome sequencing



11 total shared variants:

- 10 non-coding
- 1 coding (*GNAQ*)

- Functional annotation of 1300 variants using VAAST
- Only one variant (*GNAQ* 548G→A) was identified as deleterious

GNAQ somatic mutations are associated with uveal melanomas and melanocytic lesions

- Occurs sporadically
- Lesions are distributed in a mosaic pattern
- Variable extent of involved tissue
- Localized - phenotype does not spread
- Sex ratio is 1:1
- Sounds familiar...



Lee, C.-W. et al. (2005). An infantile case of Sturge-Weber syndrome in association with Klippel-Trenaunay-Weber syndrome and phakomatosis pigmentovascularis. *Journal of Korean medical science*.

GNAQ mutations in uveal melanoma

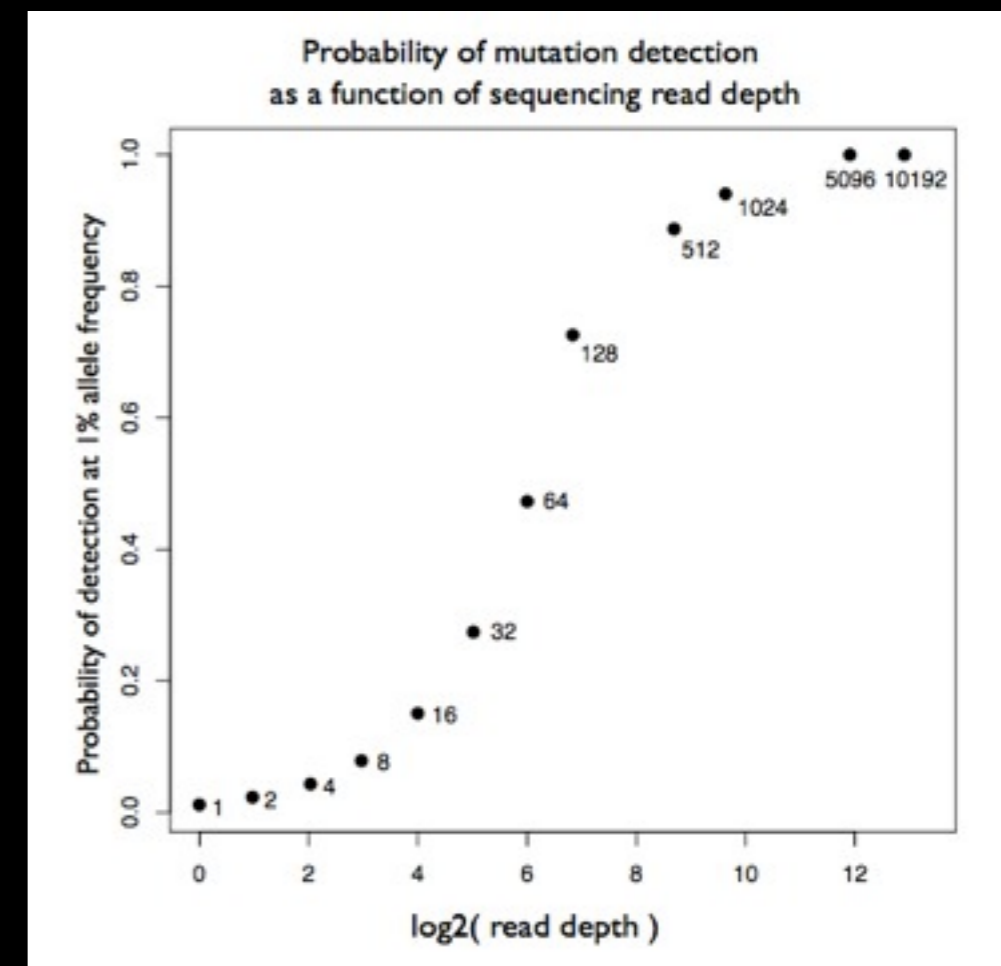
- Q209L mutation is most common
- R183Q (548G→A) mutation is less frequent
- Both also cause non-cancerous melanocytic lesions (blue nevus and nevus of Ota)
- Rare co-occurrences of SWS and melanocytic lesions are reported

Robae, Al. et al. (2004). Phakomatosis pigmentovascularis type IIb associated with Sturge-Weber syndrome. *Pediatric Dermatology*.

Validation by deep amplicon sequencing

Validation by deep amplicon sequencing

- Custom PCR amplicon sequencing strategy on Illumina MiSeq
- Multiplexed with error-correcting Hamming7,4 DNA barcodes
- Error correction allowed us to decrease multiplexing failures: ~9% greater depth
- Targeted 10,000 and achieved 2,446 to 93,008 (median 12,947) read depth



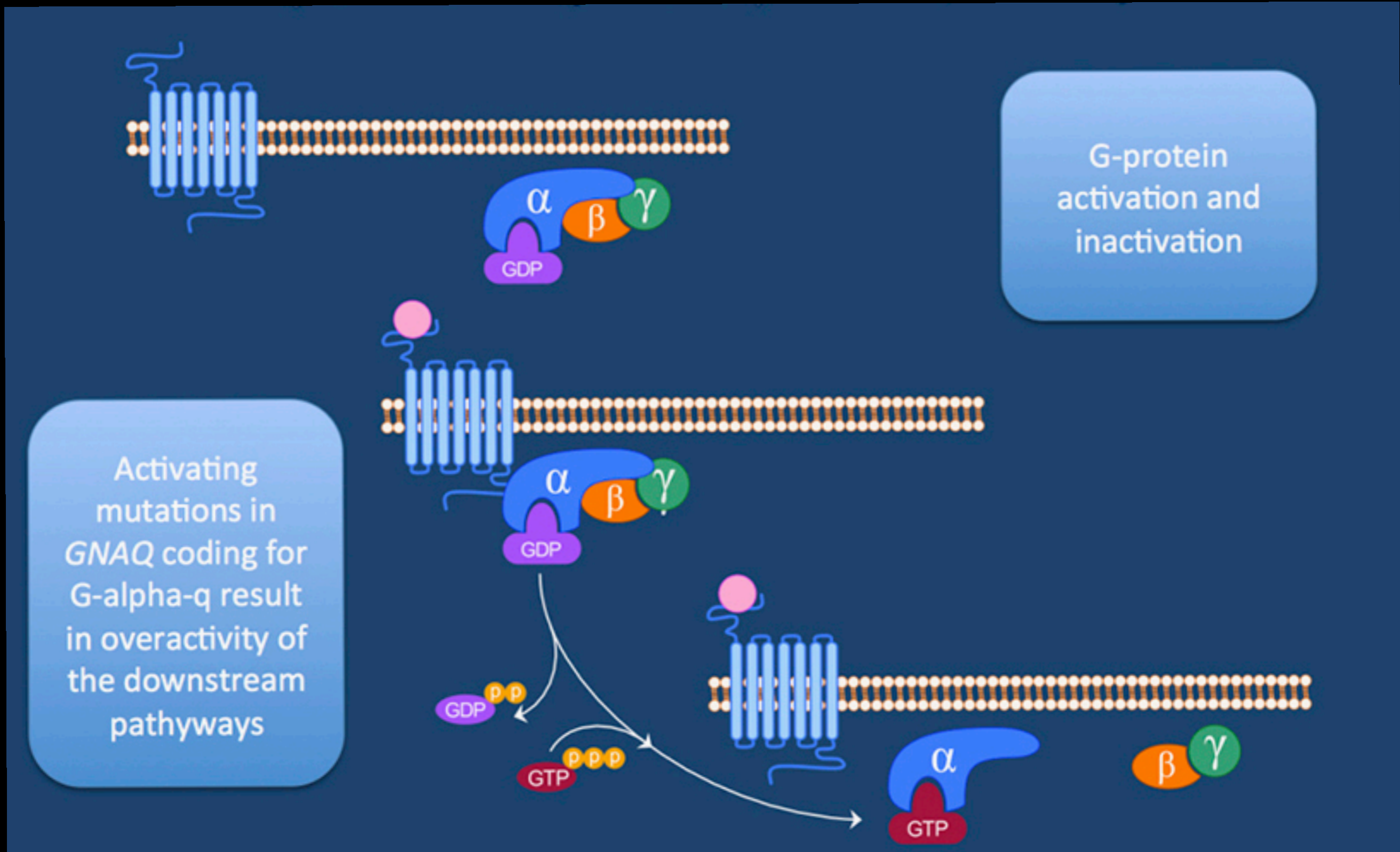
Validation by deep amplicon sequencing

#Subjects	Tissue	SWS	GNAQ R183Q	Method
9	PWS	Yes	100%	Amplicon
7	Skin	Yes	14.00%	Amplicon
13	PWS	No	92.00%	Amplicon
18	Brain	Yes	88.00%	Amplicon
6	Brain	No	0%	Amplicon
4	Brain	CCM	0%	SNaPshot
669*	Blood/LCL	N/A	0.700%	Exome

* >271X median read depth exomes from 1000 Genomes Project

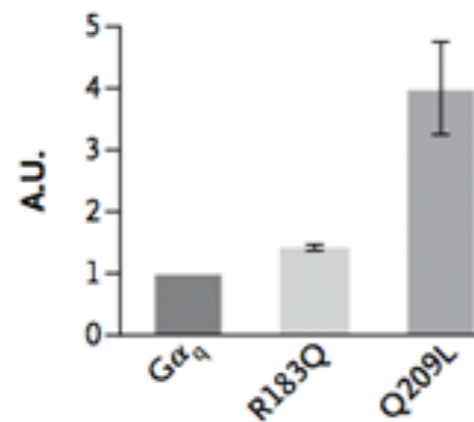
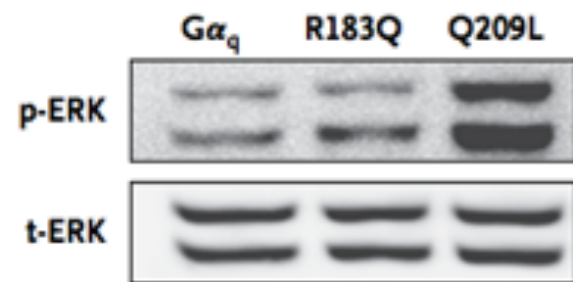
Biology of GNAQ mutations

GNAQ is a G-protein alpha subunit

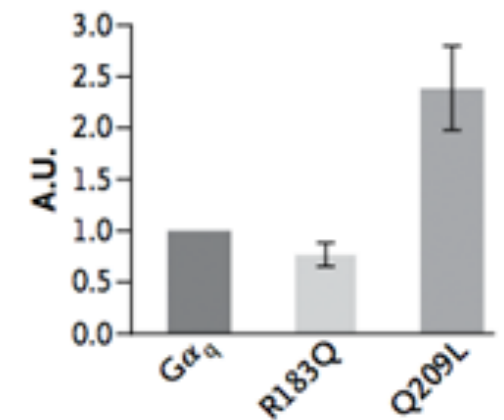
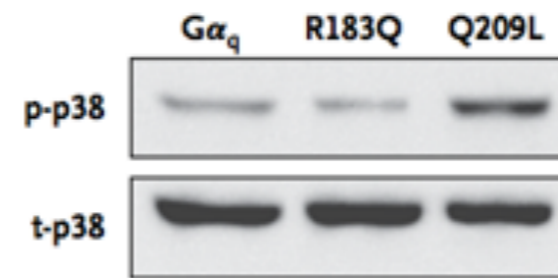


R183Q is an activating mutation

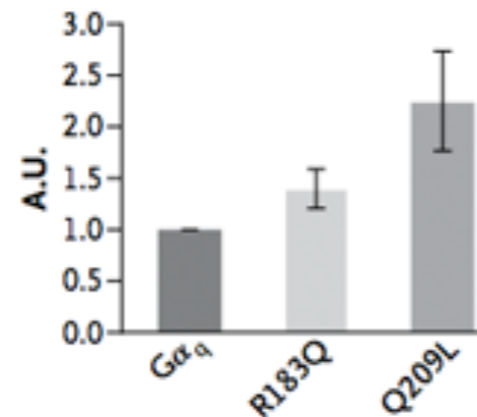
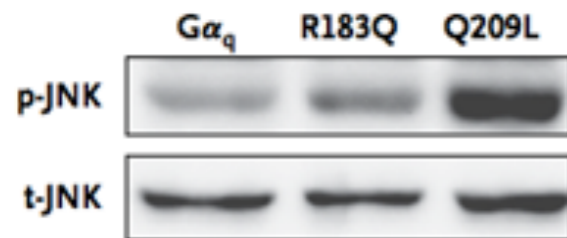
A ERK Phosphorylation



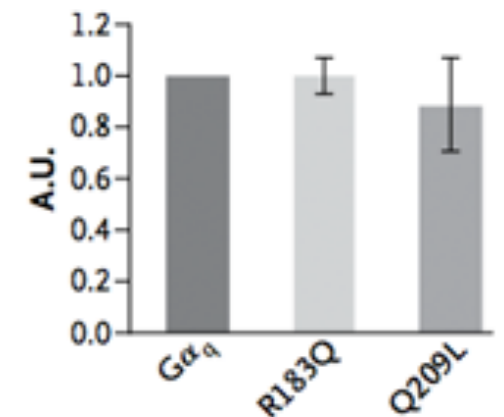
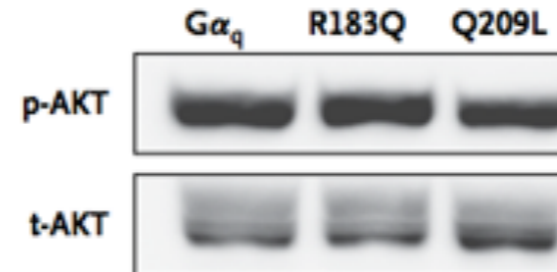
B p38 Phosphorylation



C JNK Phosphorylation



D AKT Phosphorylation



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Conclusions

- Sturge-Weber syndrome and port-wine stains appear to have one prevalent genetic basis
- GNAQ R183Q mutations activate downstream MAPK pathways
- Carefully chosen subject and sample populations combined with deep and broad sequencing allows rapid discovery of rare disease variants

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