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

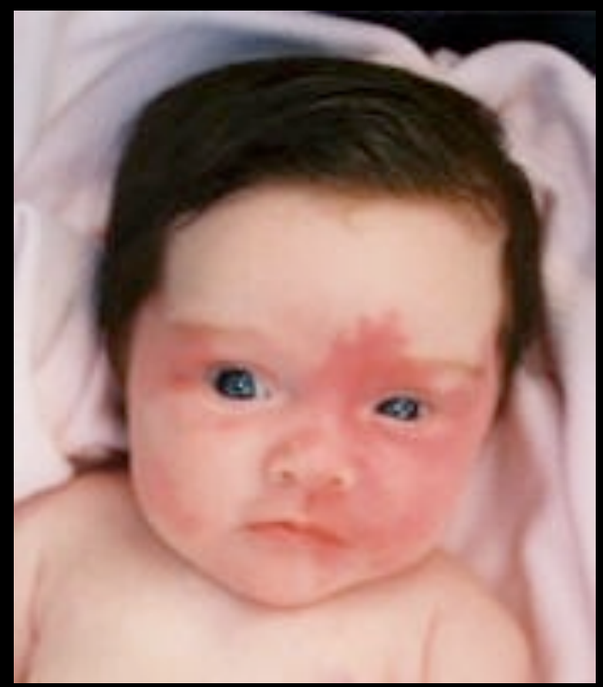
Matt Shirley, Ph.D.
Department of Oncology
Yegnasubramanian / Wheelan labs

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

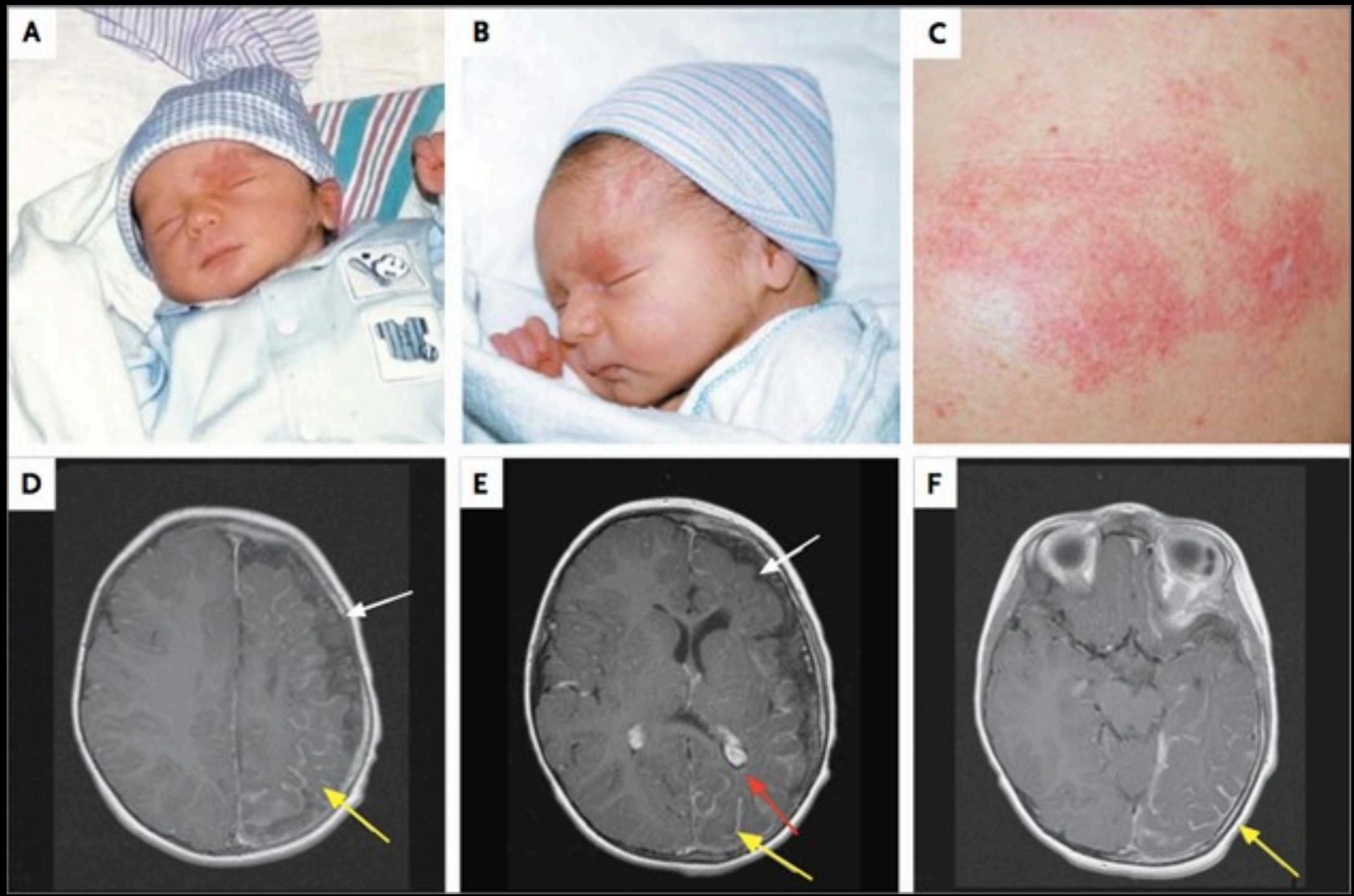
Pathology of the Sturge-Weber syndrome

- 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 development[1]
- Happle was proven correct for McCune-Albright[2] (*GNAS*) and Proteus[3] (*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:
  AAAAAAAAAAAAAAAABBBBBBBBBBBBBB
- Signal (B) is greater than noise (N)
  AAAAAAAANAAAAAAAAABBBBBBBBBBBNBB
  = 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:

  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAABB

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

  AAAAANAAAAAAAAANAAAAAAAAAAAAABB

  = 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

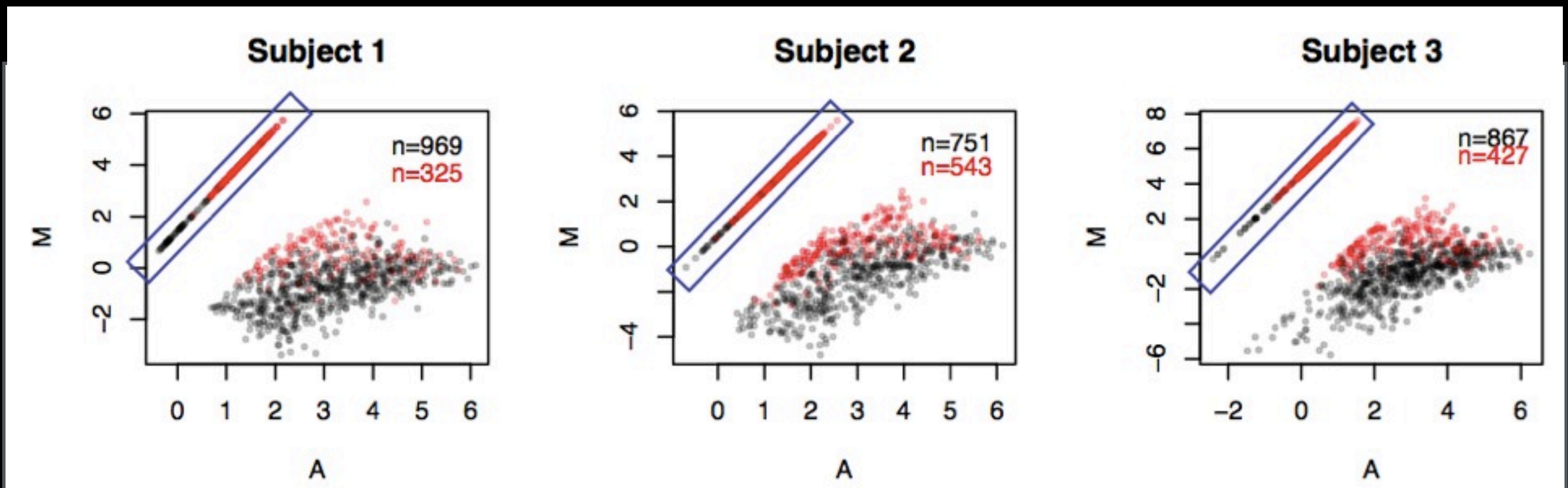
- Achieved 33-51X average coverage in 6 paired samples from 3 subjects
- Matched normal / affected tissue samples
- Strelka somatic genotyper

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

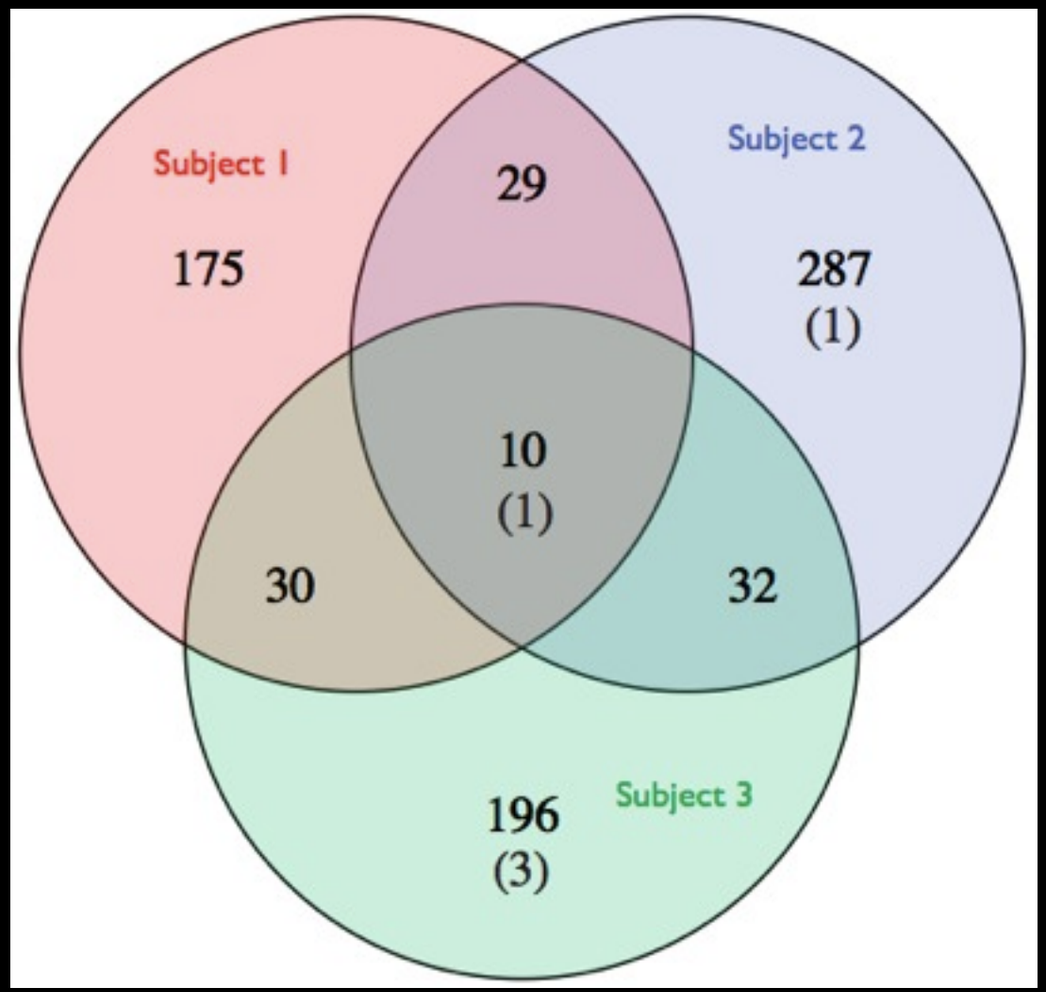
- Concerned about missing low frequency somatic variants
- Look at all call sites for any alternate alleles in affected (absent normal)



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



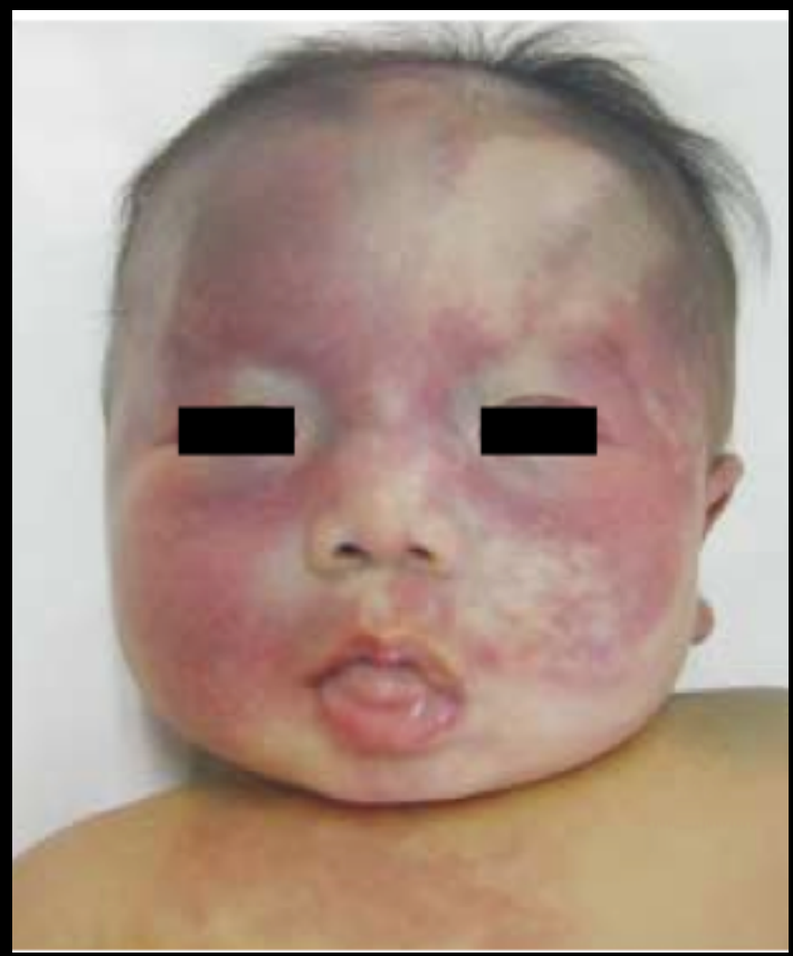
11 total shared variants:

- 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

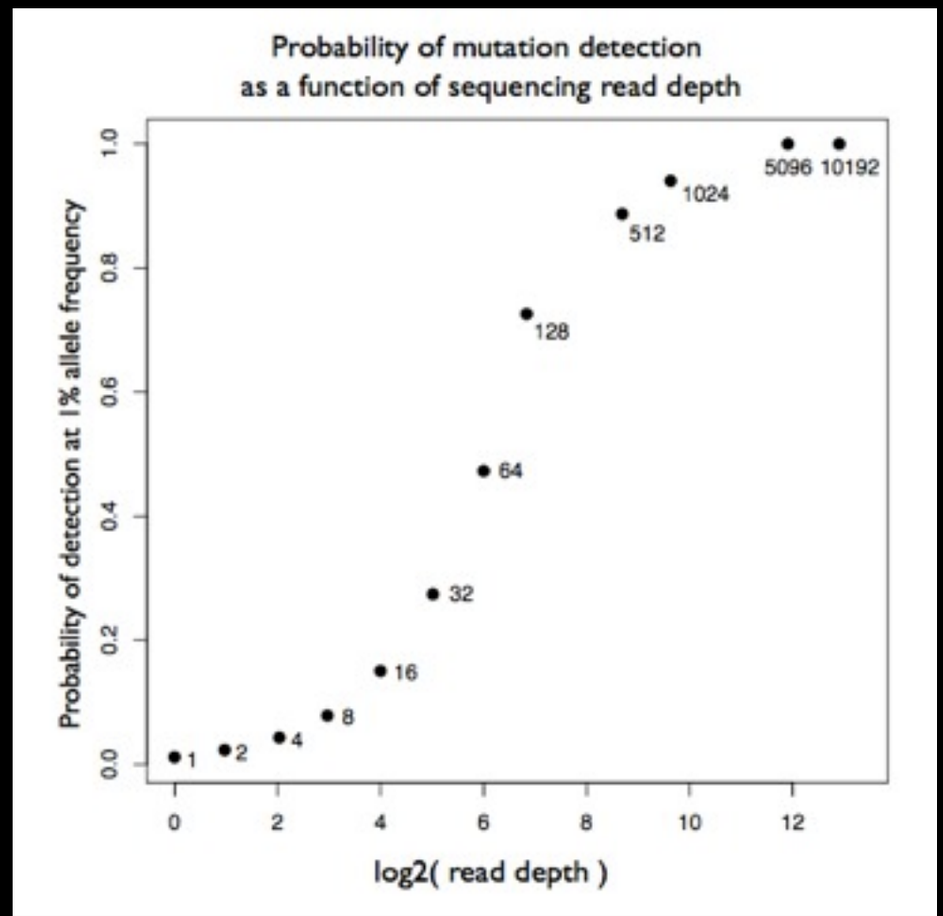
- Q209L mutation is most common
- R183Q (548G→A) mutation is less frequent
- Both also cause non-cancerous melanocytic lesions (blue nevus and nevus of Ota)
- Rare co-incidences of SWS and melanocytic lesions are reported

Robaee, 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



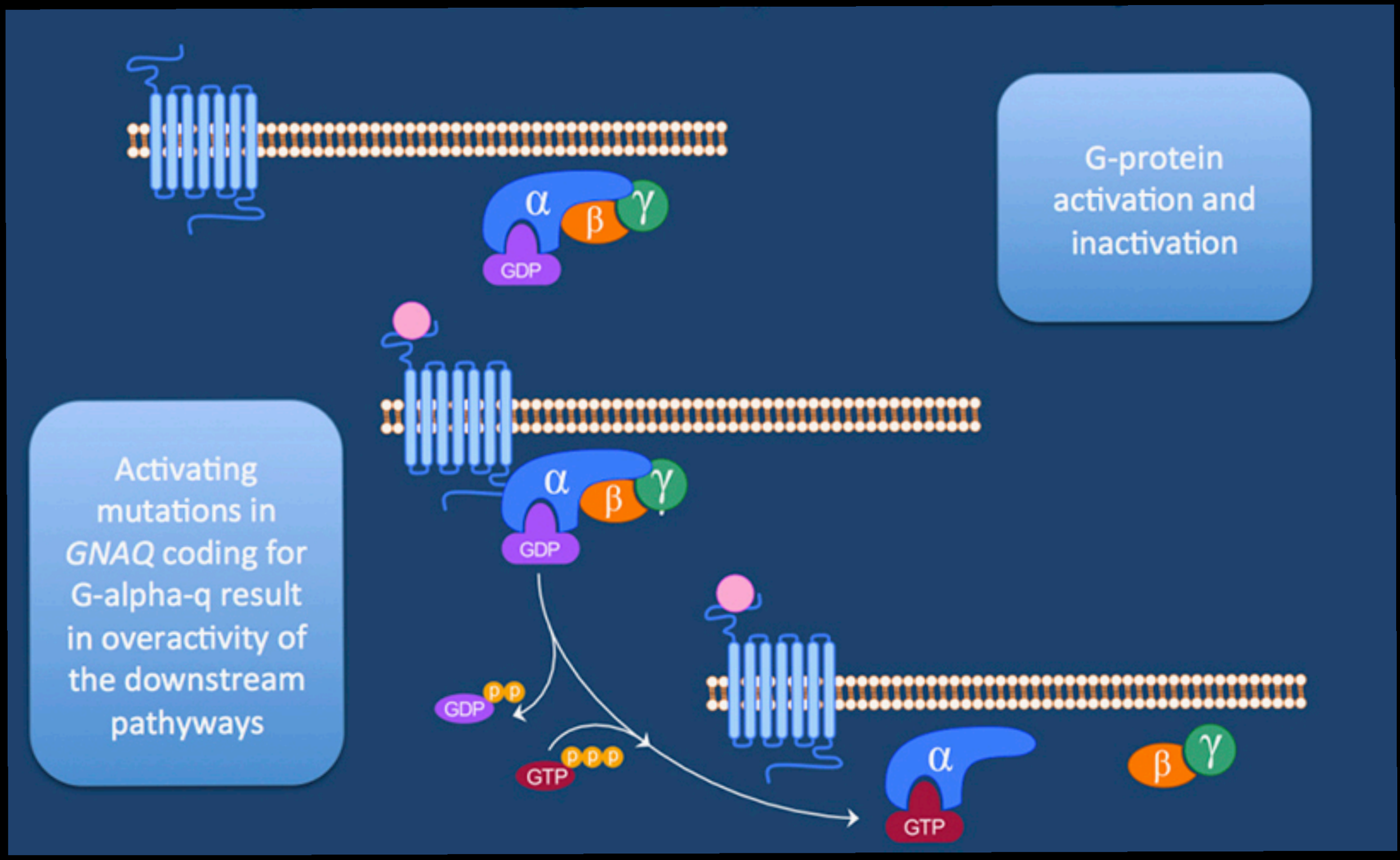
Validation by deep amplicon sequencing

#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

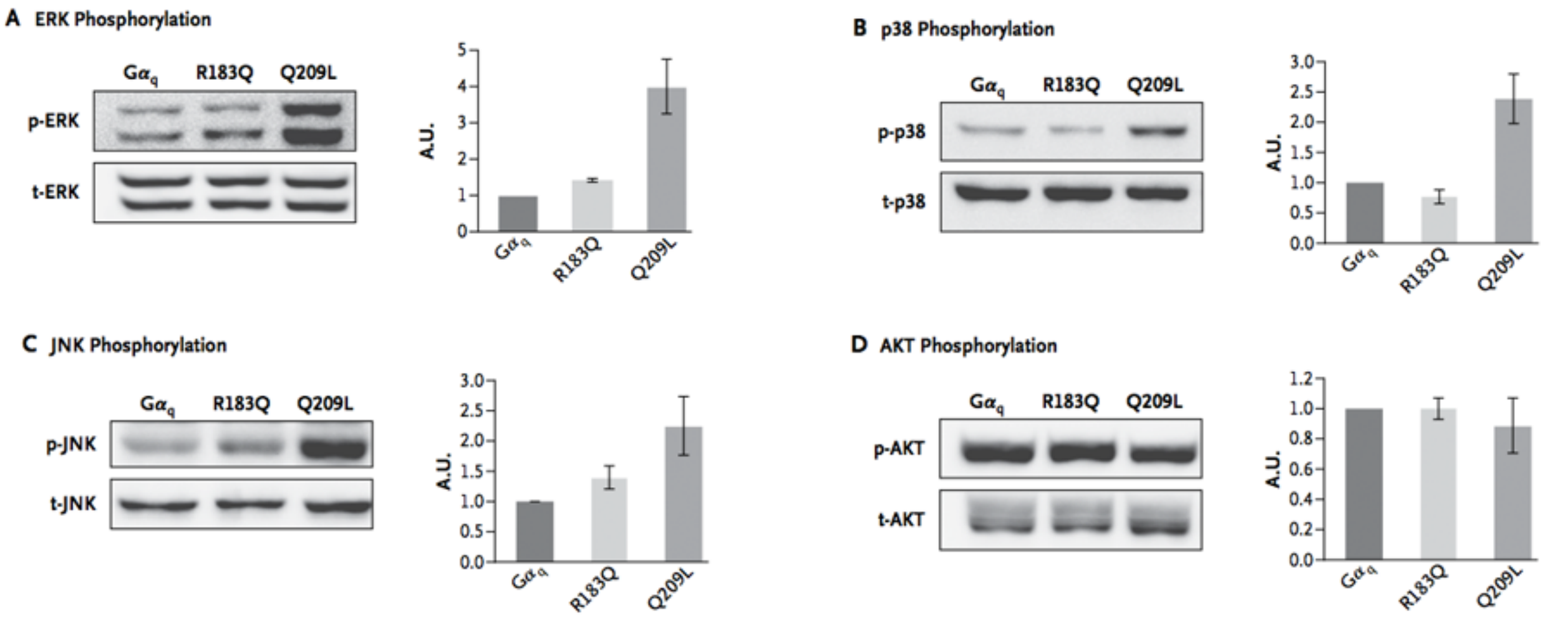
* >271X median read depth exomes from 1000 Genomes Project

Biology of GNAQ mutations

GNAQ is a G-protein alpha subunit



R183Q is an activating mutation



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

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

Acknowledgements

- Jonathan Pevsner
- Doug Marchuk
- Anne Comi
- Eric Stevens
- Joe Baugher
- Larry Frelin
- Sarah Wheelan
- Vasan Yegnasubramanian
- Gary Cutting
- Dani Fallin
- Sturge-Weber patients
- Hunter's Dream for a Cure Foundation
- National Institute of Neurological Disorders and Stroke (NINDS)