BRIGHAM AND WOMEN'S HOSPITAL

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The Many Faces of Mosaicism in TSC

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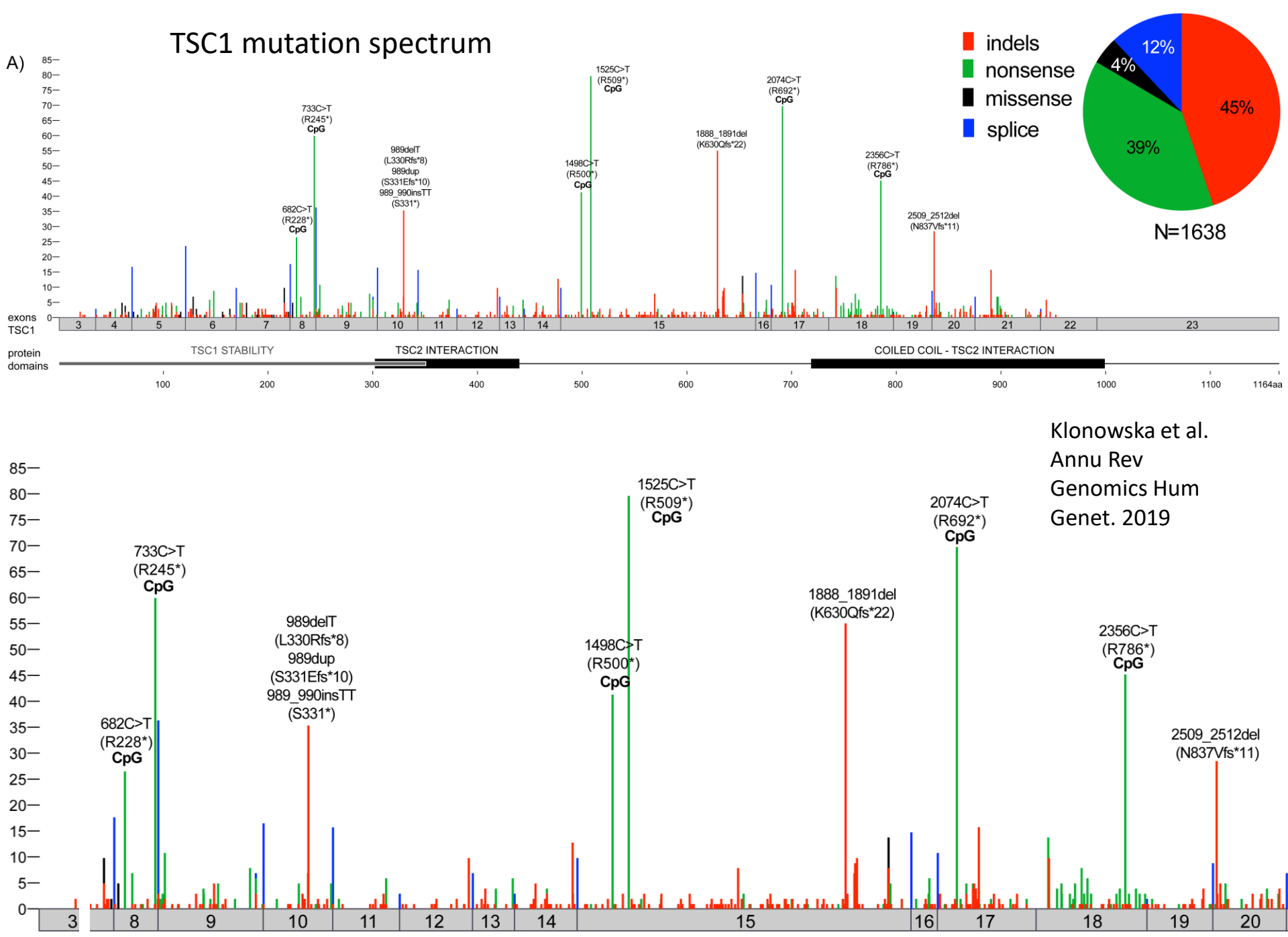
TSC clinical genetics

- Incidence - 1 in 6-10,000;
  -> ~40,000 Americans with TSC

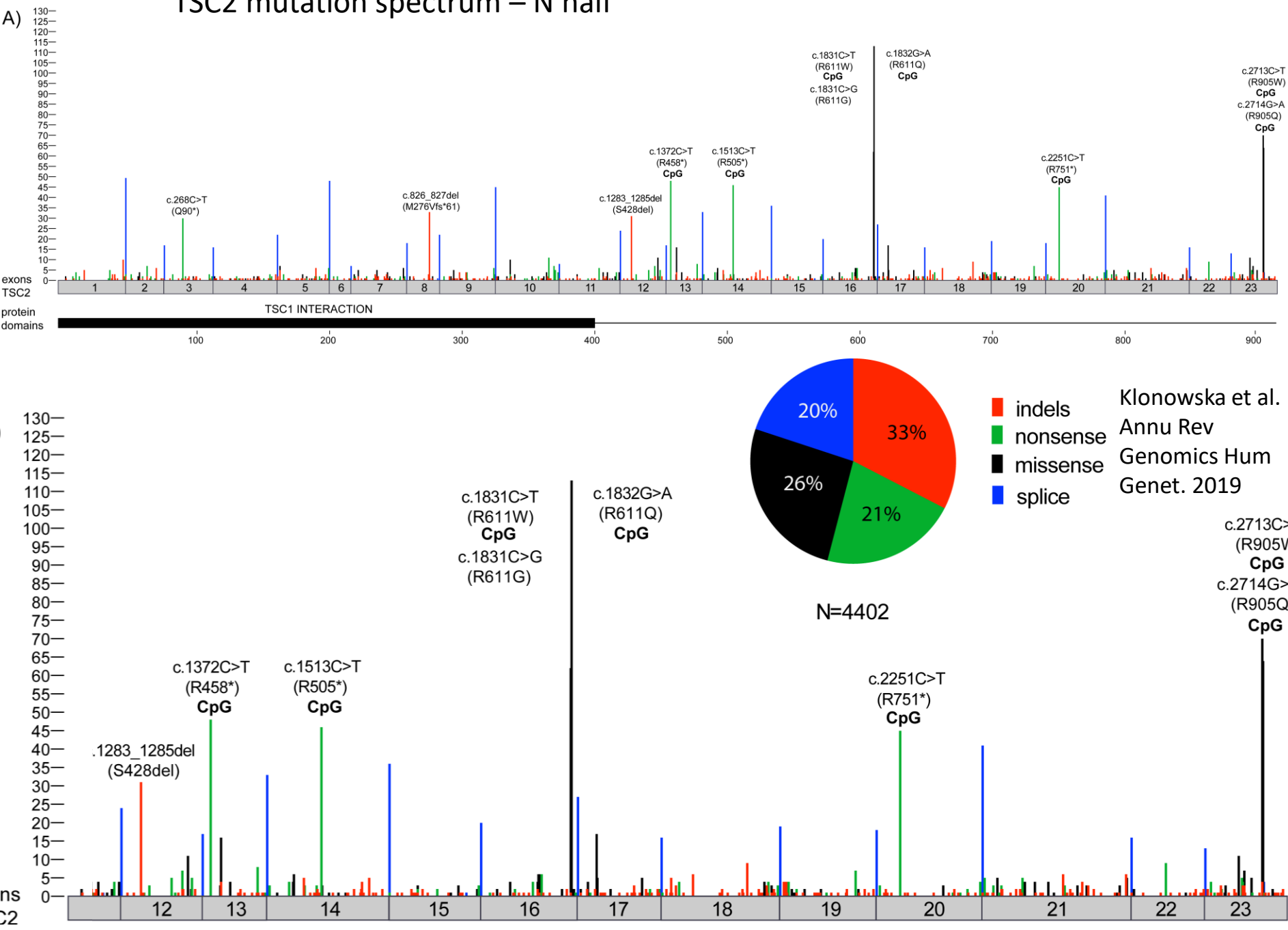
- Autosomal dominant inheritance – means each TSC individual (without mosaicism) has a 50% chance of transmitting TSC to each of their children

- Variable expression of the disease
  - but very rarely skips a generation
  - some rare families have mild features

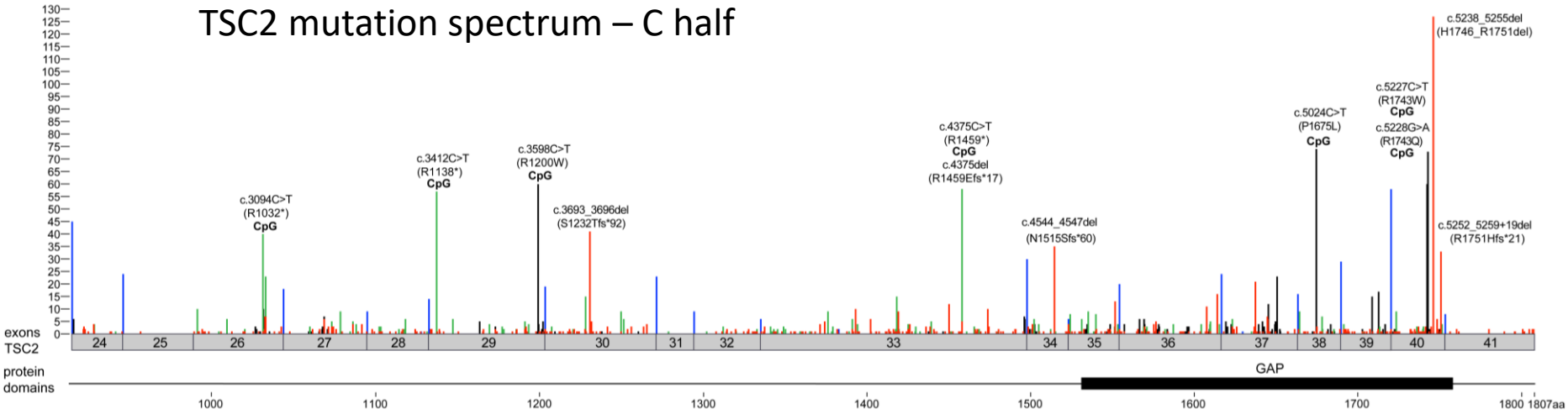
- Sporadic cases (no family history) account for about 2/3 of all patients

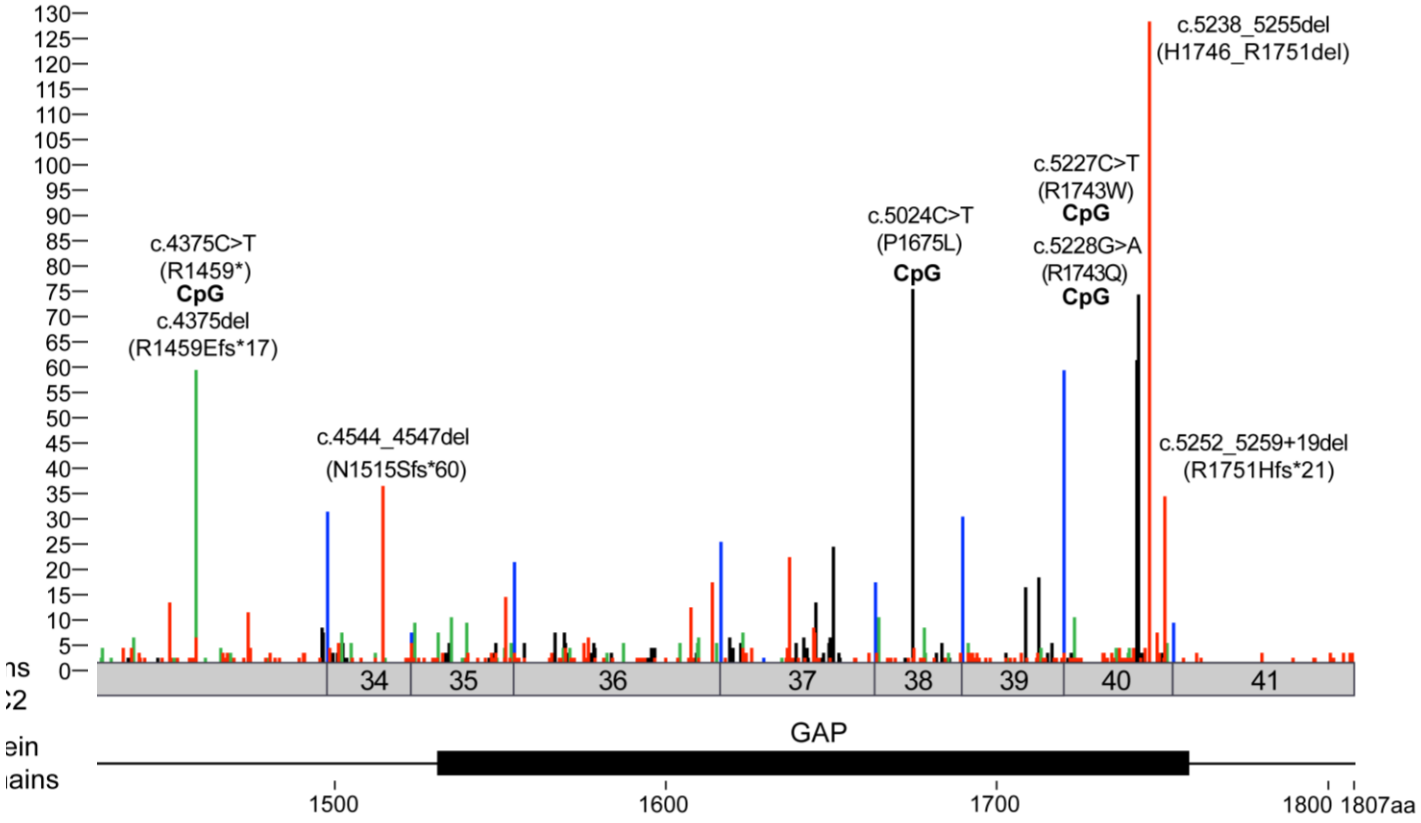

TSC1 mutation spectrum
A)
indels
nonsense
missense
splice
45%
39%
4%
12%
N=1638
733C>T (R245*) CpG
682C>T (R228*) CpG
989delT (L330Rfs*8) 989dup (S331Efs*10) 989_990insTT (S331*)
1525C>T (R509*) CpG
1498C>T (R500*) CpG
1888_1891del (K630Qfs*22)
2074C>T (R692*) CpG
2356C>T (R786*) CpG
2509_2512del (N837Vfs*11)
exons TSC1
protein domains
TSC1 STABILITY
TSC2 INTERACTION
COILED COIL - TSC2 INTERACTION
1164aa
Klonowska et al.
Annu Rev
Genomics Hum
Genet. 2019

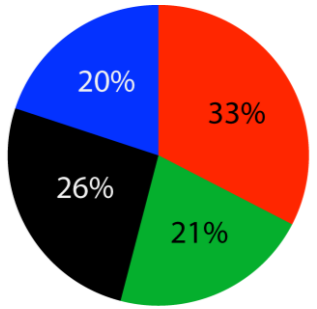
TSC2 mutation spectrum – N half



TSC2 mutation spectrum – C half





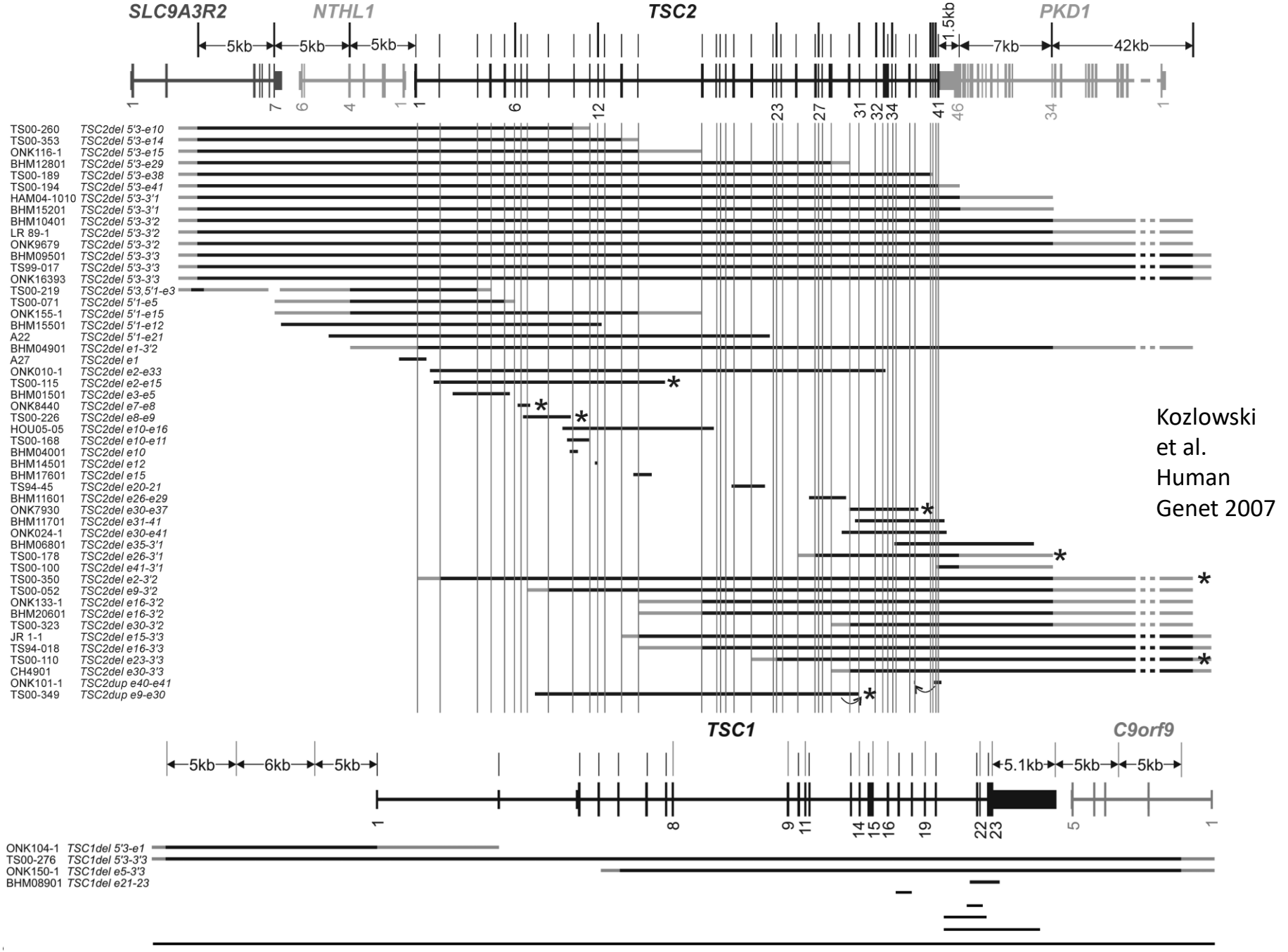


N=4402

- indels
- nonsense
- missense
- splice

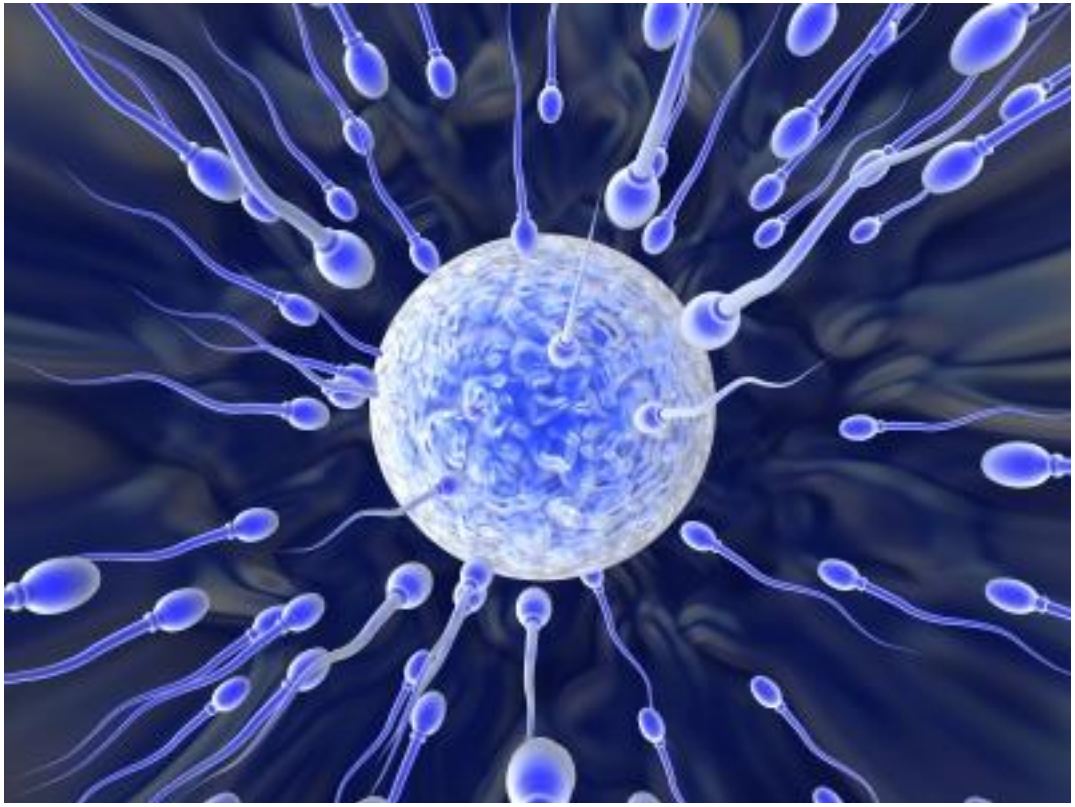
Klonowska et al.
Annu Rev
Genomics Hum
Genet. 2019

TSC2 genomic deletions ~5%, TSC1 genomic deletions ~1%, of all TSC



Kozlowski et al. Human Genet 2007

Fertilization leads to the first cell of a new embryo

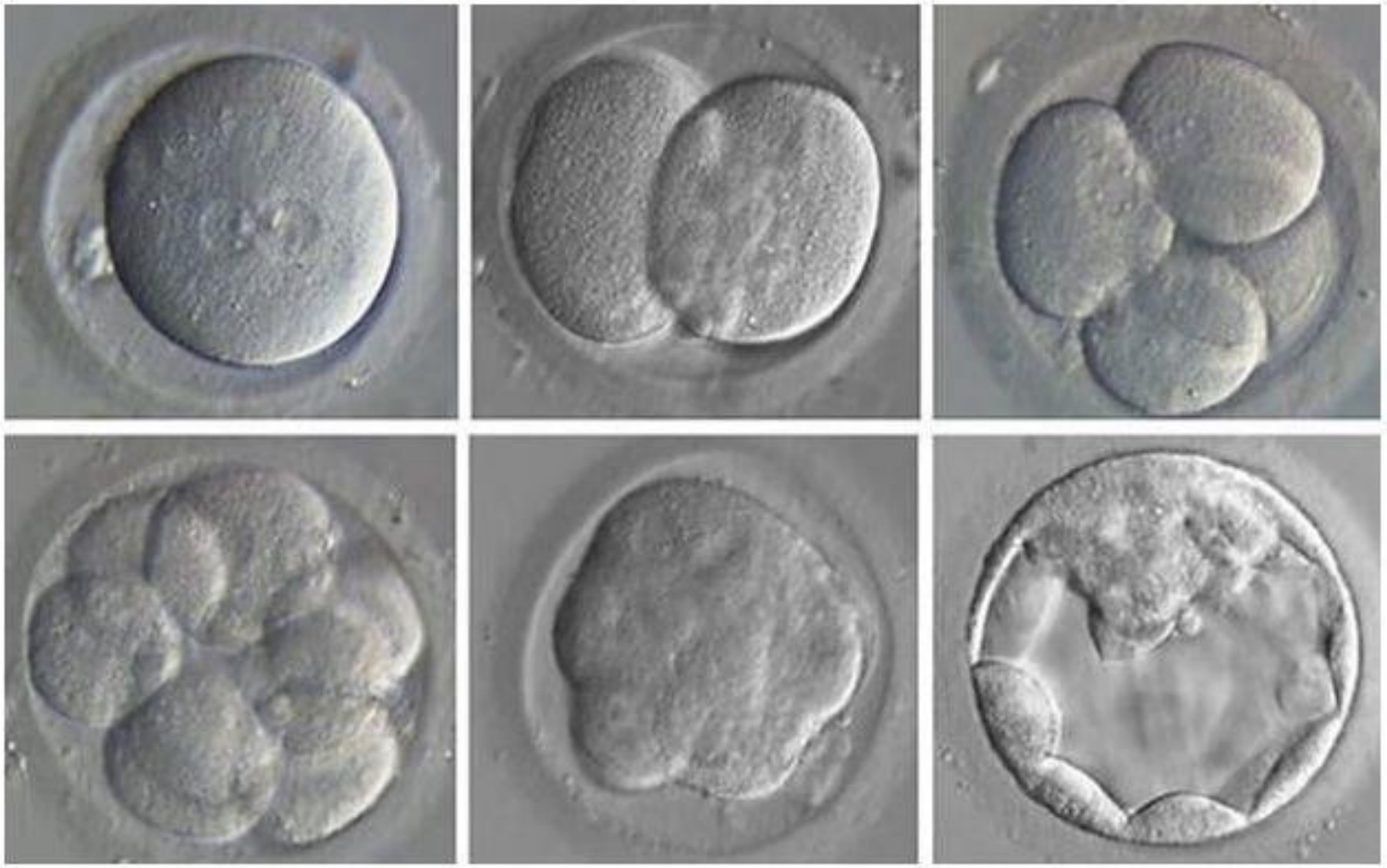


Fertilization is followed by serial divisions that lead to all the cells in the body

1 cell

2 cells

4 cells

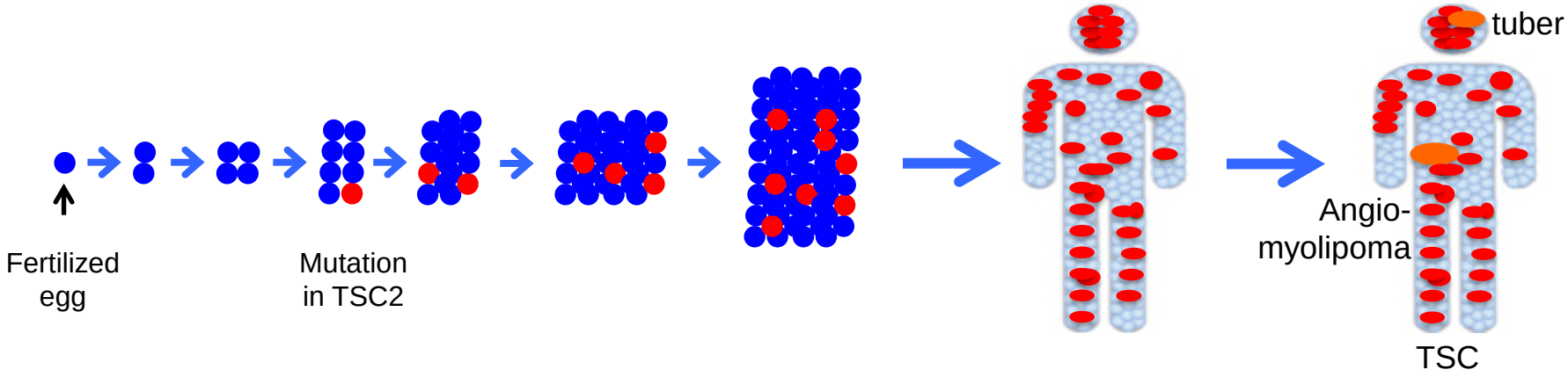


8 cells

~15 cells

~100 cells

Mosaicism is common both in general and in TSC

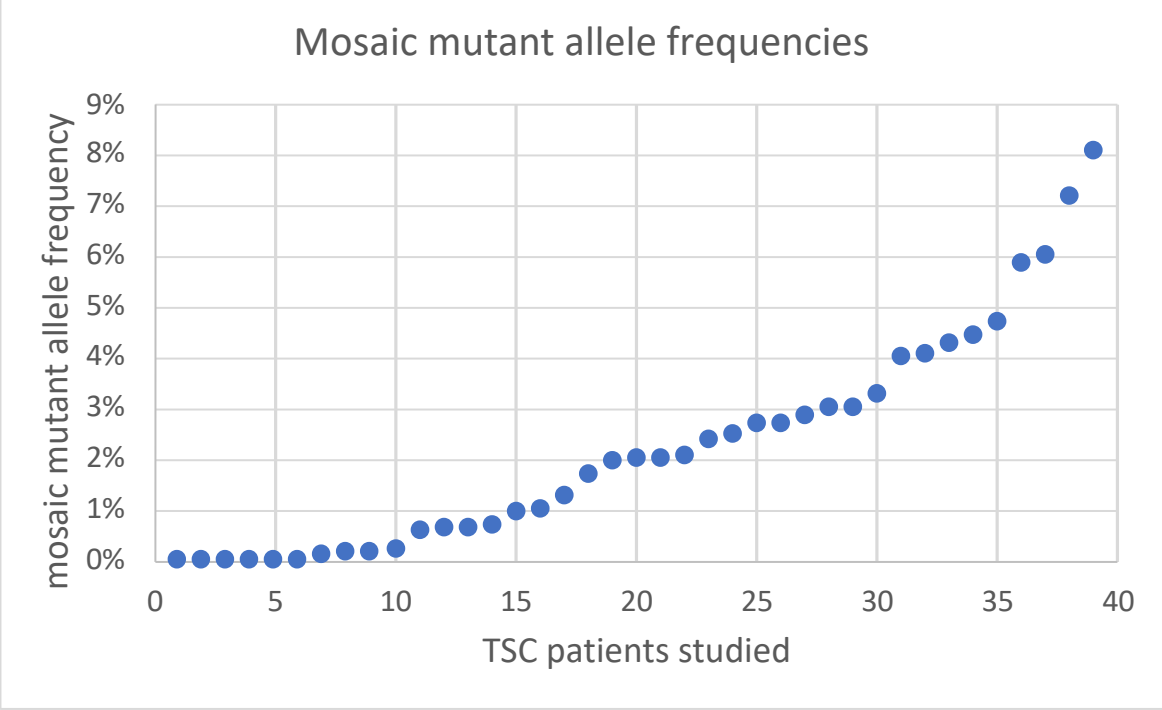


PLOS | GENETICS 2015

RESEARCH ARTICLE

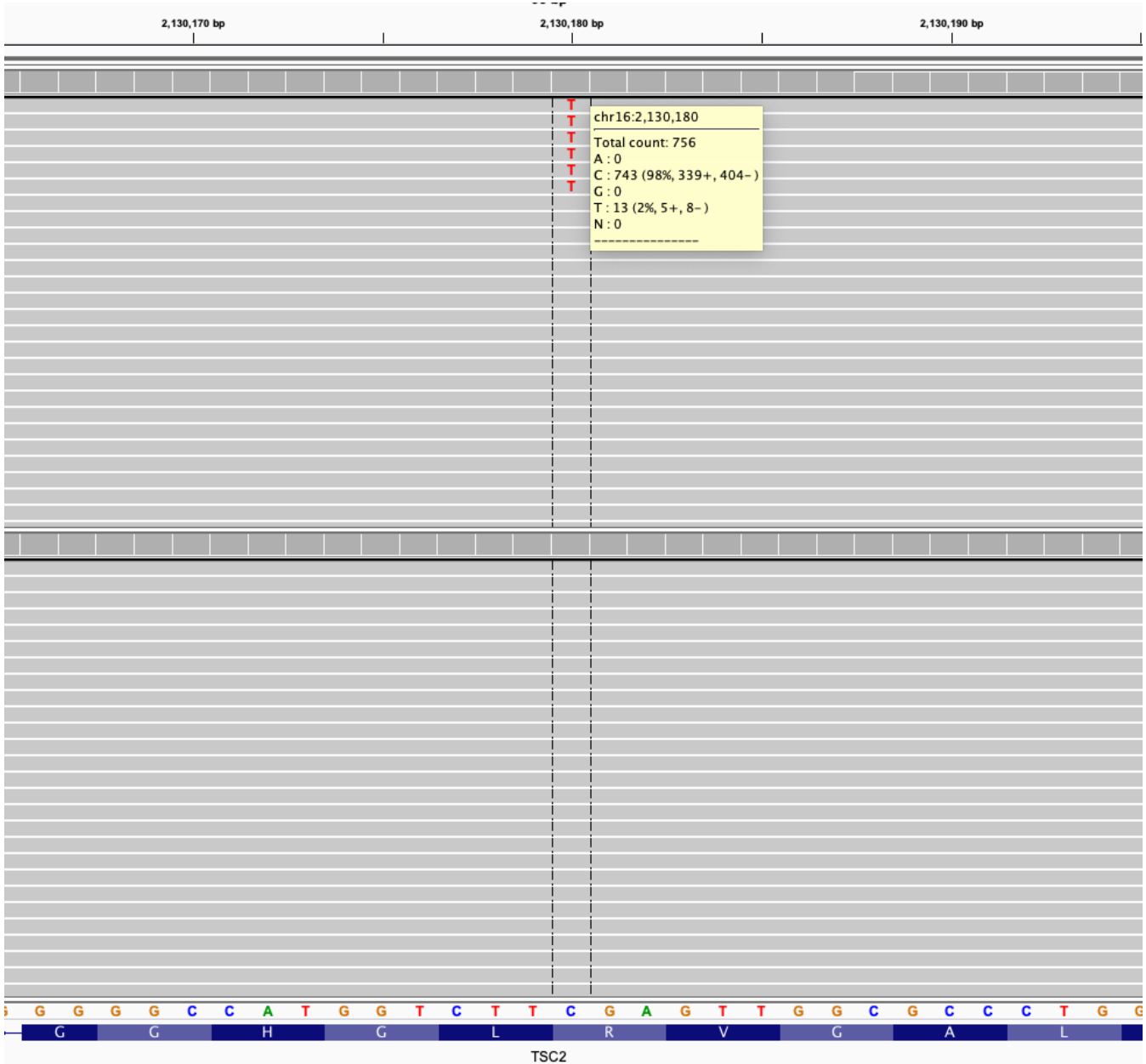
Mosaic and Intronic Mutations in *TSC1/TSC2* Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing

Magdalena E. Tyburczy[1], Kira A. Dies[2], Jennifer Glass[3], Susana Camposano[4], Yvonne Chekaluk[1], Aaron R. Thorner[5], Ling Lin[5], Darcy Krueger[6], David N. Franz[6], Elizabeth A. Thiele[4], Mustafa Sahin[2], David J. Kwiatkowski[1]*



Giannikou et al.
Genet in Med 2019

How do we find mosaic variants?



Sample from a TSC individual
Note 756 total reads
13 of them (1.7%) show a sequence variant
C>T
Which creates a stop codon, TGA

Similar analysis of a control shows no reads with this sequence variant

The Appearance of Mosaicism

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Dermatology
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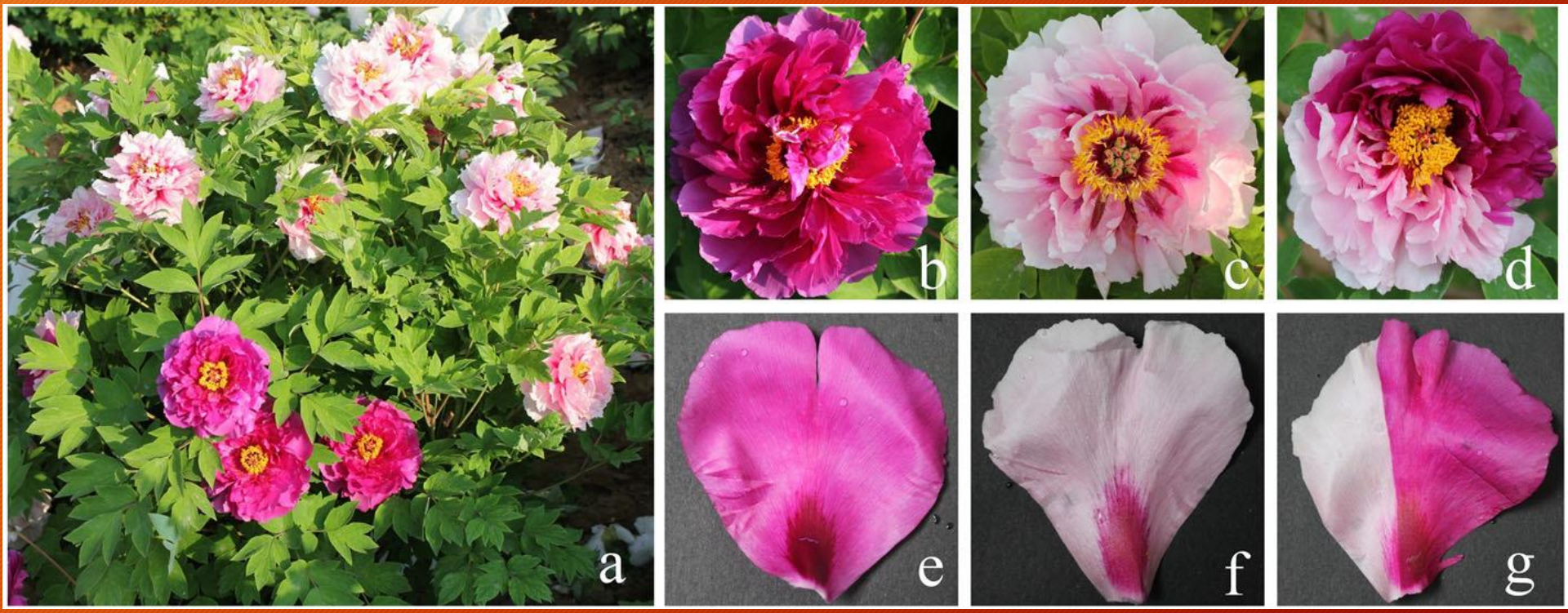
Disclaimer

- The opinions and assertions expressed herein are those of the author and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

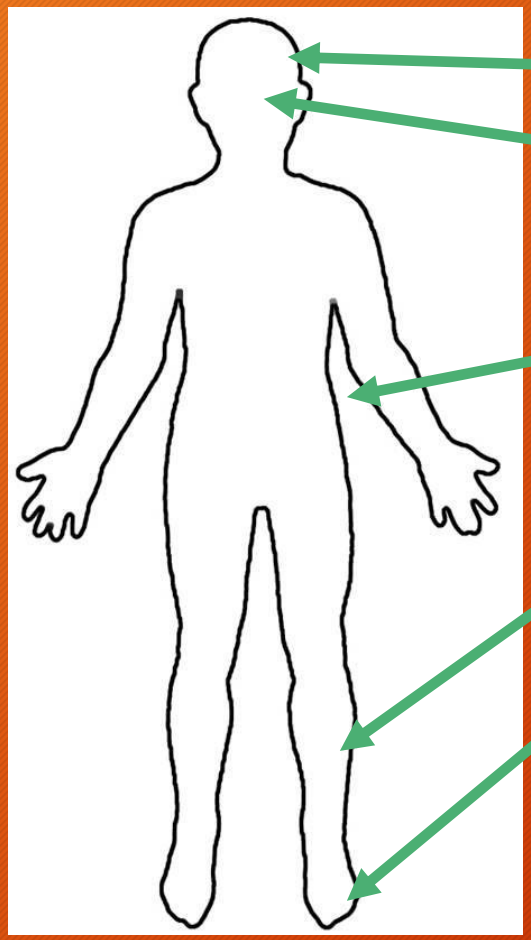
- Neither I nor my family members have a financial interest in any commercial product, service, or organization providing financial support for this research.

Mosaicism in plants



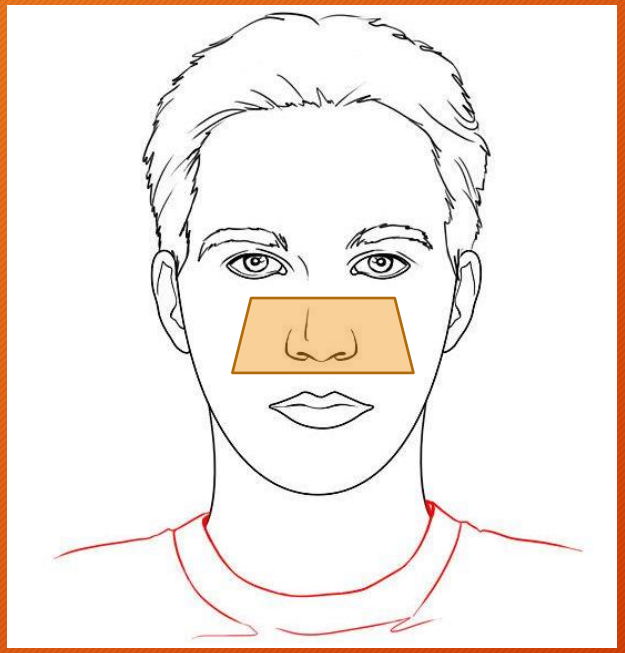
BMC Plant Biol. 2020 May 12;20(1):211

Changes in the skin in TSC

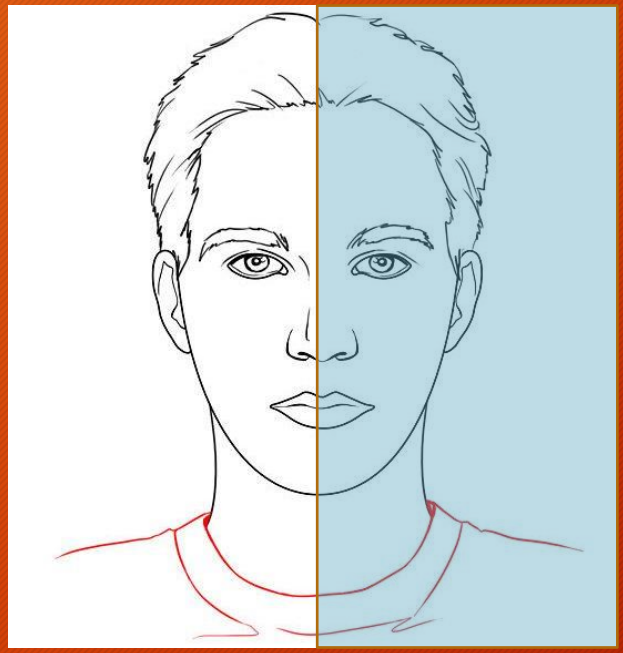


- Fibrous Cephalic Plaque
- Angiofibromas
- Shagreen Patch
- Hypomelanotic macules
- Ungual fibromas

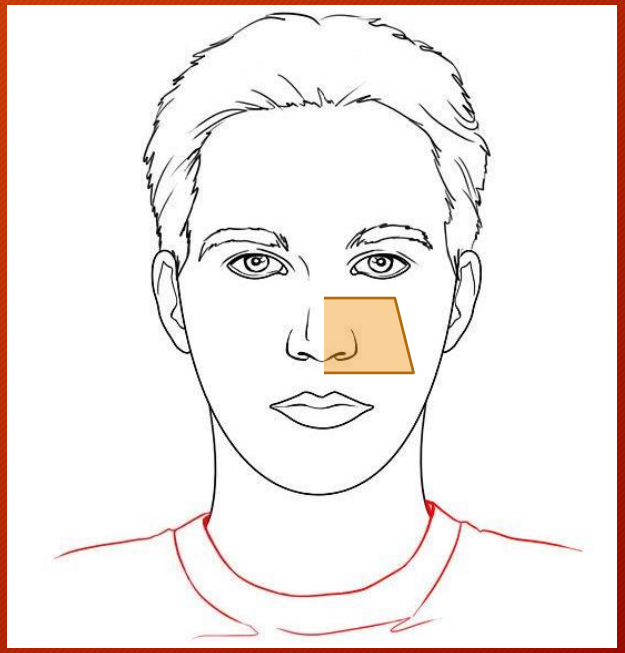
Asymmetrical Facial Angiofibromas



Germline

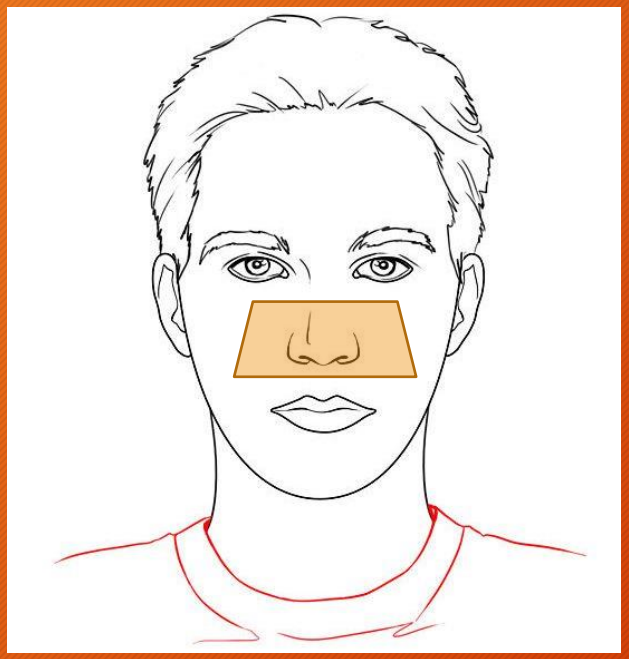


Left sided Mosaicism

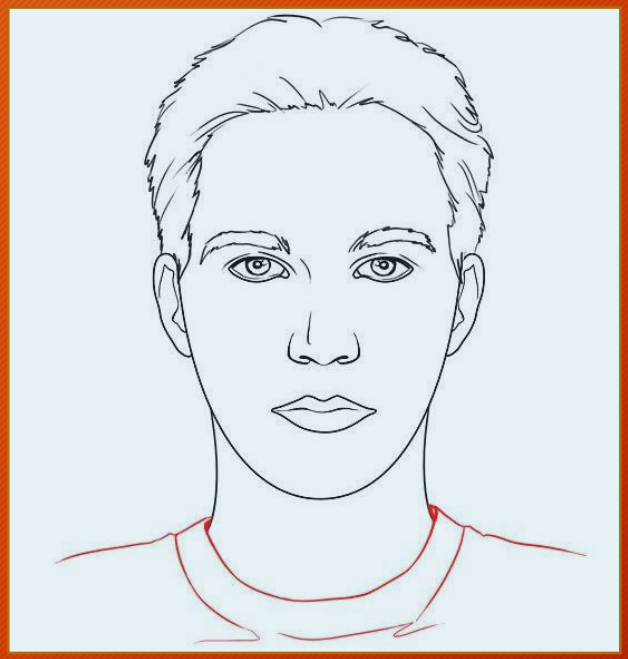


Mosaic

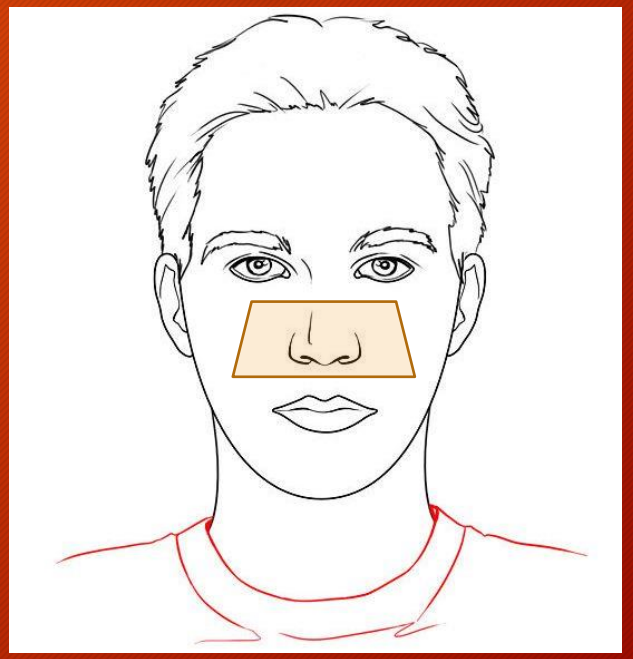
Symmetrical Facial Angiofibromas



Germline

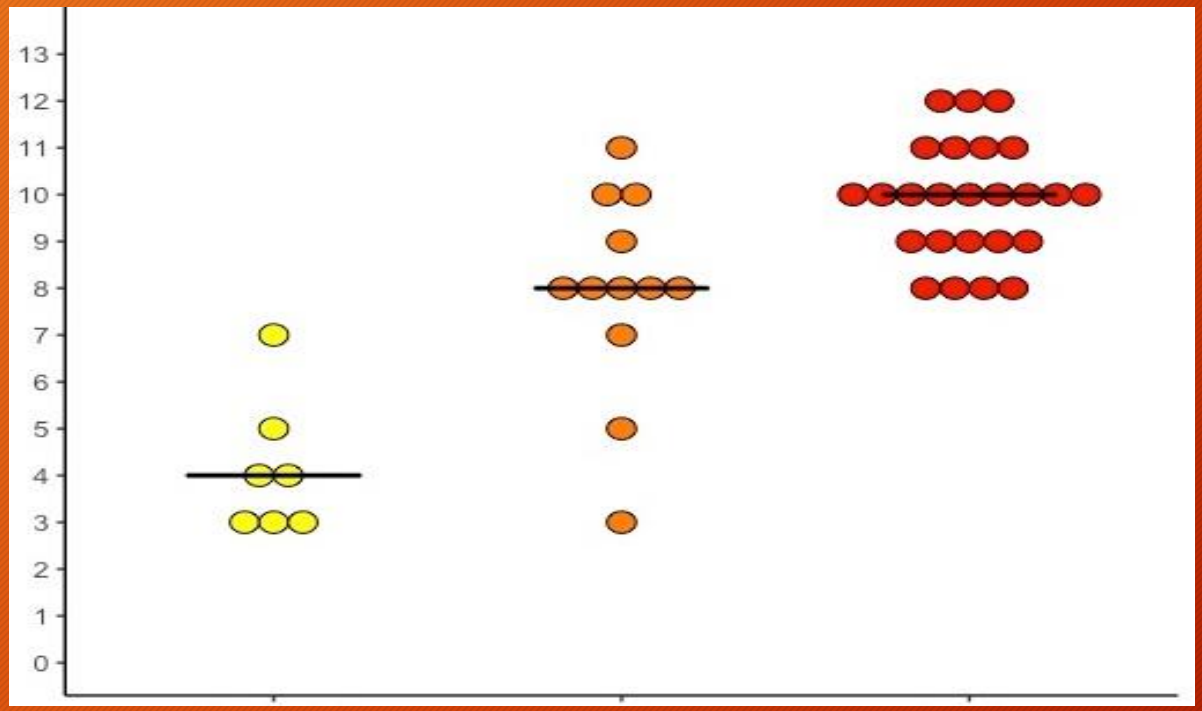


Generalized Mosaicism



Mosaic

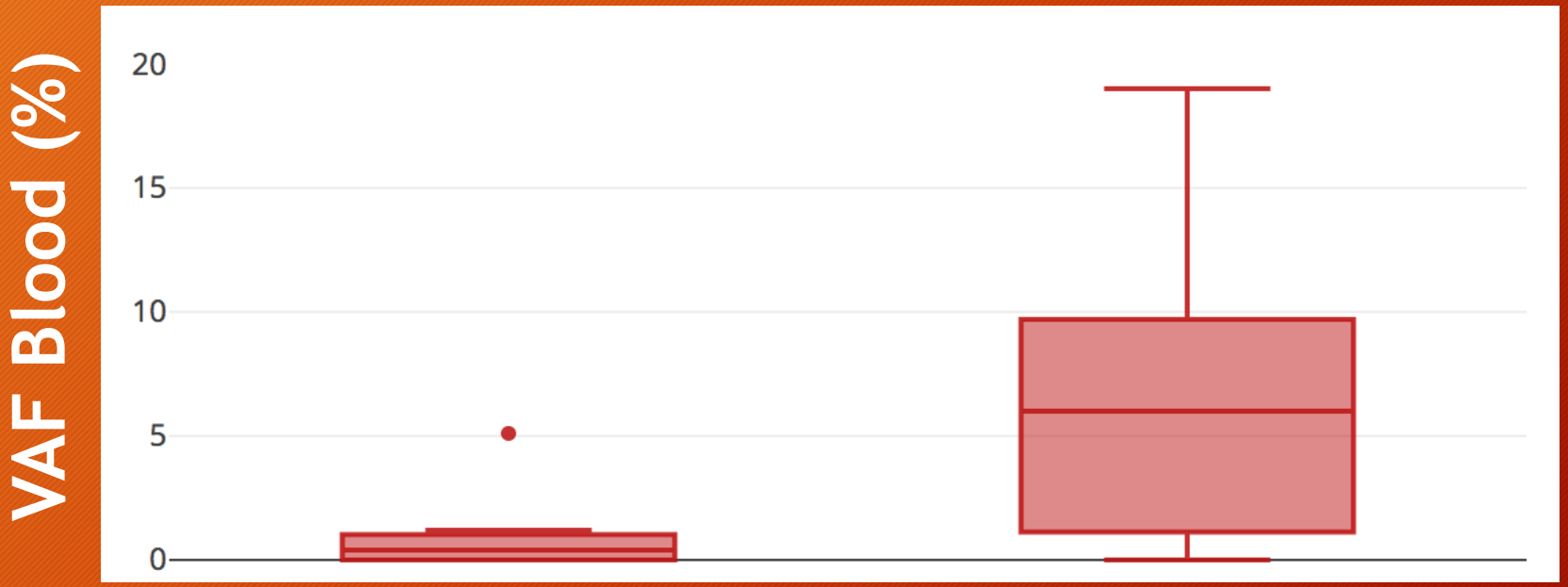
Less severe disease with mosaicism



Also later onset:
Asym-AF – 24 yrs
Sym-AF- 10 yrs
Germline - 4 yrs

Genet Med. 2019;21(11):2594-2604

Mosaicism detection in blood



Genet Med. 2019;21(11):2594-2604

Skin samples for genetic analysis

- First demonstration of mosaicism in TSC using a skin sample was in 2011

  Nat Commun. 2011;2:235

- Done initially using cells grown in the laboratory

  Hum Mol Genet. 2014 ;23(8):2023-9

- More readily done with DNA extracted directly from skin biopsy

  PLoS Genet. 2015;11(11):e1005637
  Genet Med. 2019;21(11):2594-2604
  Genet Med. 2019 ;21(11):2639-2643

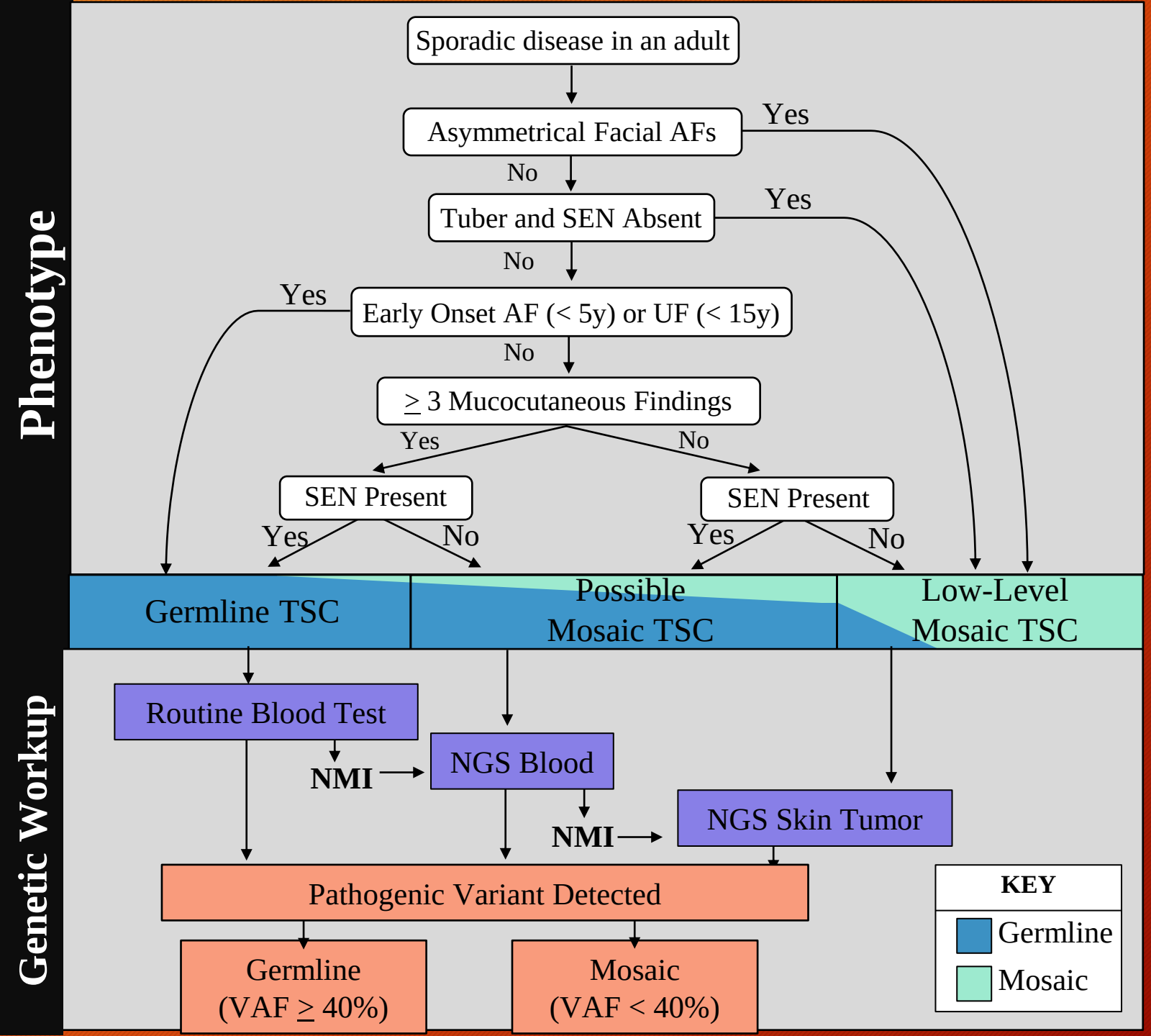
Other findings that may suggest mosaicism

- No tubers or subependymal nodule (SEN)
  - 11 patients with TSC and no tubers or SEN. 10 had *TSC1/TSC2* mutational analysis, which was negative. Hypothesized mosaicism.

    Clin Genet. 2014;86(2):149-54
  - 3 patients with no tubers or SEN, all mosaic.

    Genet Med. 2019;21(11):2594-2604
- No sclerotic bone lesions (SBL)
  - 92 adult patients with TSC. SBL in 82 (89%). Patients without bone lesions had negative mutational studies of *TSC1/TSC2* in 86%.

    Am J Med Genet A. 2017;173(7):1891-1895



Br J Dermatol. 2020;182(1):235-237

Summary

- Skin samples are useful for genetic analysis, particularly in those with clinical features of TSC but negative results using blood
- Some with mosaicism may be indistinguishable from those with germline disease
- Asymmetrical AFs are a marker of mosaicism
- Those with asymmetrical AFs are likely to have milder disease

Unanswered questions about skin and mosaicism

- Are there additional skin findings that may serve as markers?
  - Combinations of unilateral lesions
  - Hypomelanotic macules
- Which type of skin sample is most useful for genetic testing?
  - Angiofibromas, fibrous cephalic plaque, shagreen patch, ungual fibroma
  - Shave biopsy or punch biopsy
- Can someone have mosaicism only in the skin (or another organ)?

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NHLBI

NIAMS

Dermatology Foundation

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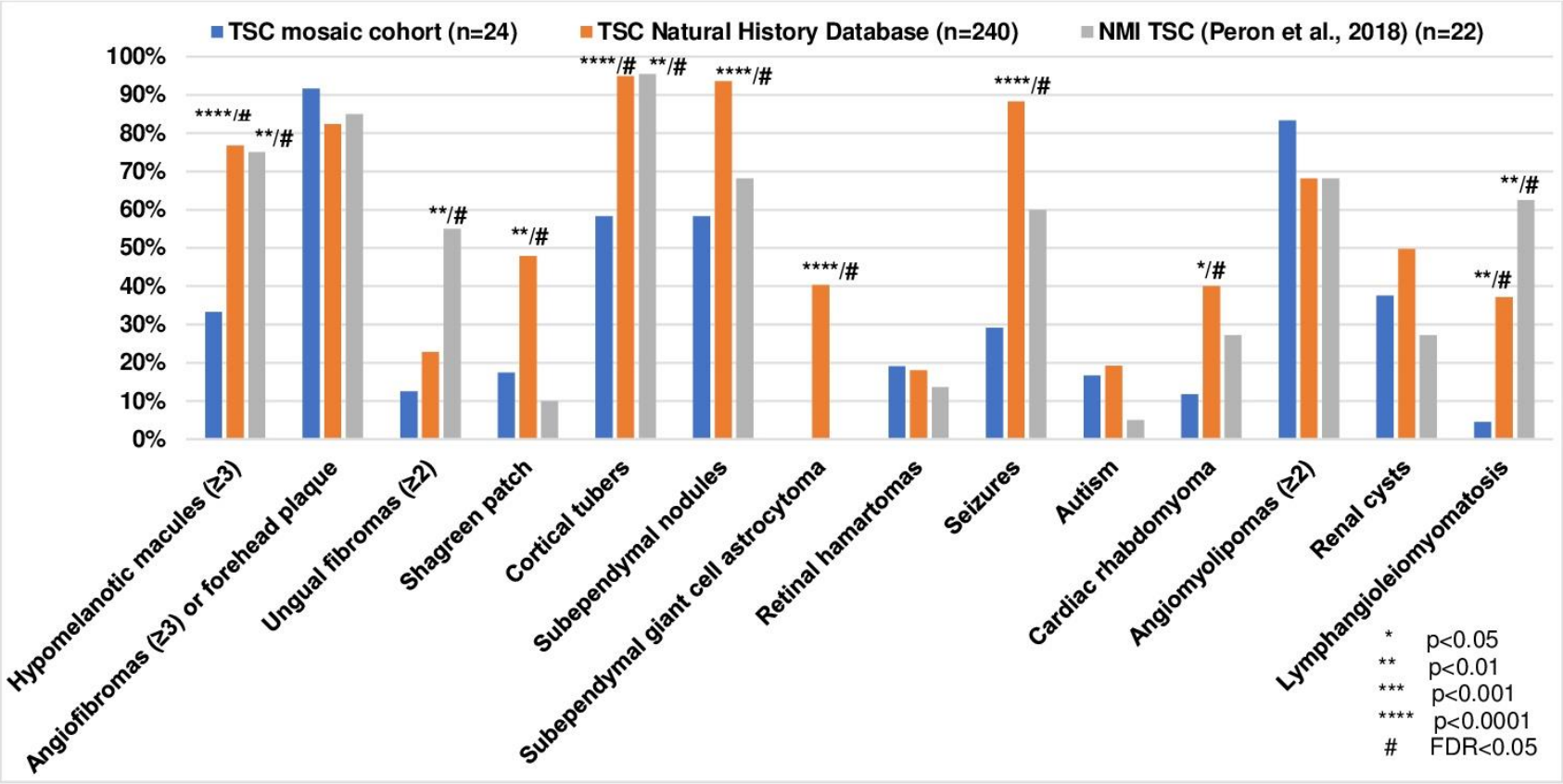
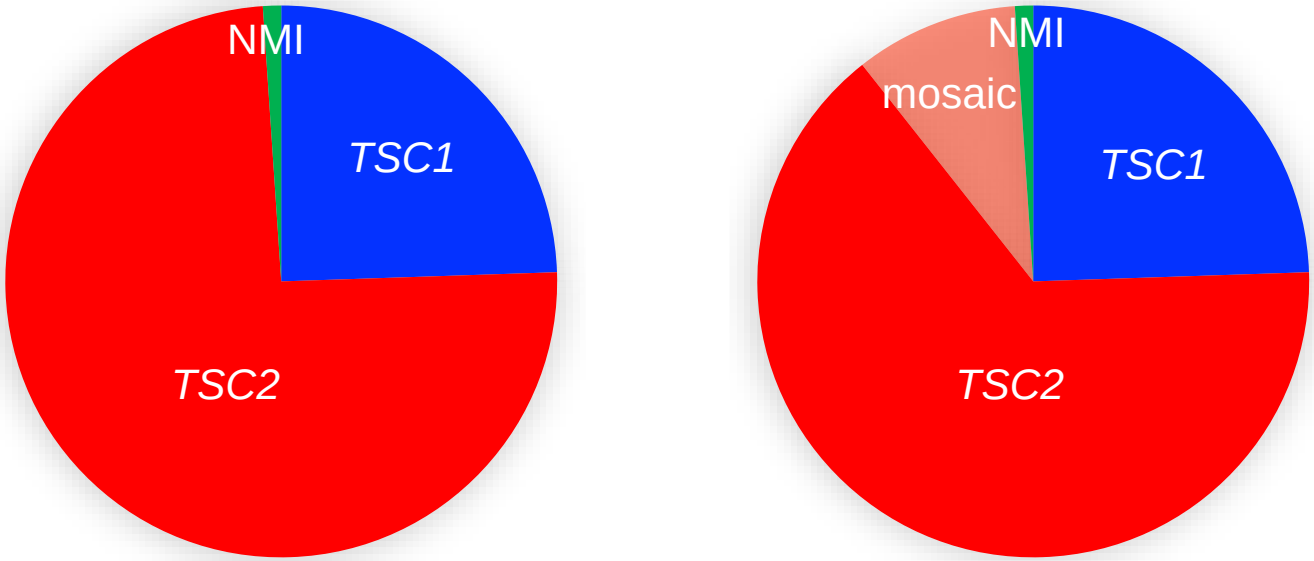
Congressionally Directed Medical Research Programs

Analysis of clinical features of 39 mosaic TSC individuals have a distinct clinical picture from those with classic TSC



Giannikou Genet Med. 2019

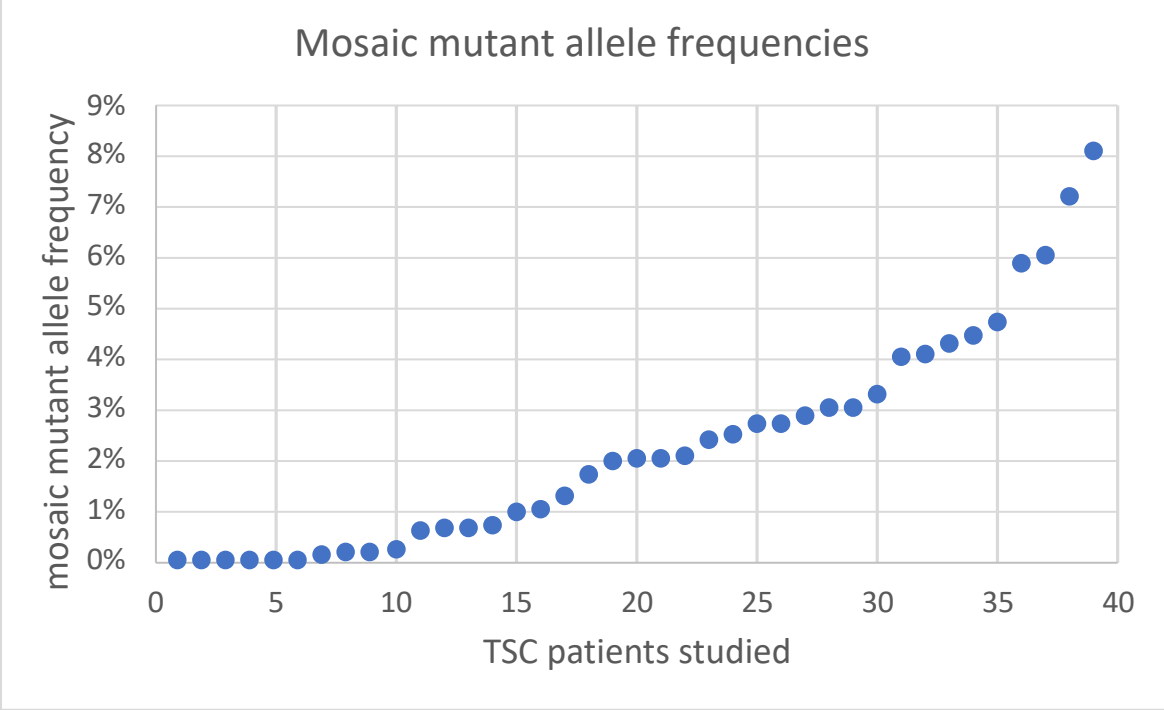
Mosaicism for a *TSC1/TSC2* gene variant is surprisingly common in EPISTOP TSC infants (n=94)



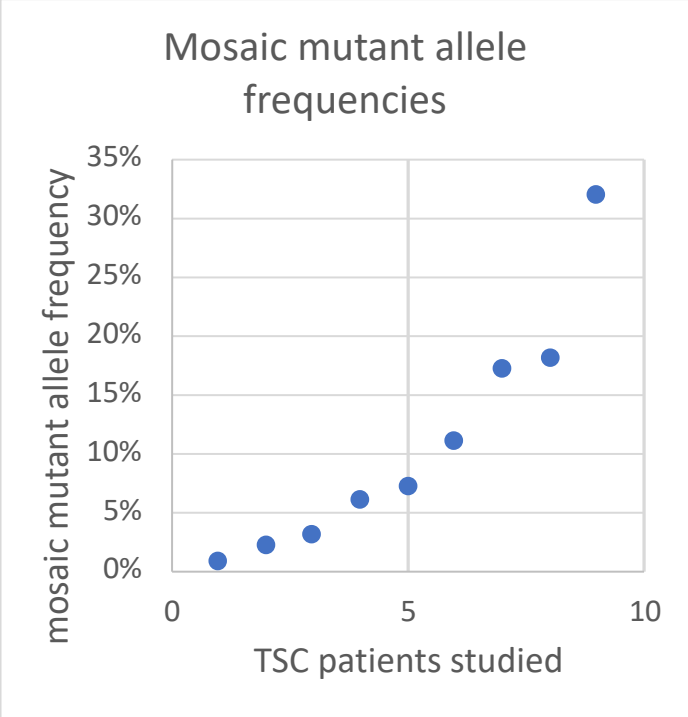
Ogorek et al. Genet in Med 2020

Level of mosaicism is much higher in the TSC infants than the adults

39 subjects, mainly young adults

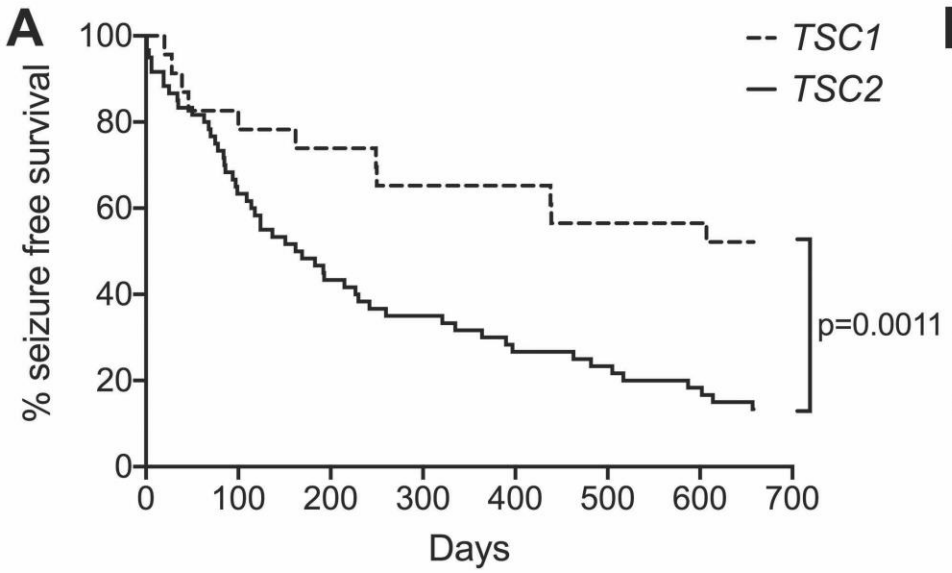


8 infants



Giannikou, Ogorek

Mosaicism in TSC infants predicts a lower risk of epilepsy, as well as other clinical features



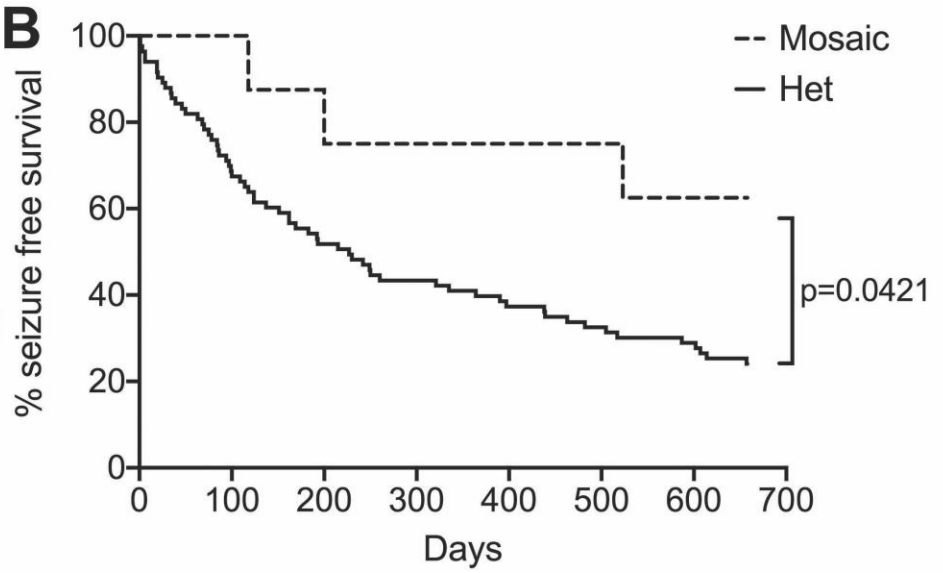


Fig.4A-B

Ogorek et al. Genet in Med 2020

Genetics of TSC and mosaicism conclusions

- Mosaicism in TSC is common (10-15%), necessitating MPS for comprehensive mutation detection. This is now standard, but most labs have an allele frequency cut-off of 2% or higher for reporting a variant

- Biopsies of skin lesions are more likely to yield a mutation finding than normal tissue samples.

- Facial angiofibroma, ungual fibromas, shagreen patch, cephalic plaque, and renal angiomyolipoma biopsies can all be used for mutation analysis, including old biopsies in many cases.

- The mosaic allele frequency in gonadal cells determines the risk of transmission of TSC for mosaic individuals. Allele frequency in gonadal cells can be assessed in men with mosaic TSC, but this is impossible in women at this point in time.

- We’re all mosaics for TSC1, TSC2, and many other gene mutations to some extent. This is likely to underlie sporadic cancer development.

Tuberous Sclerosis Complex (TSC) – a spectrum of disease

More severe
Not mosaic
TSC2 mutation
>90% seizures
infantile spasms
> 50% autism/neurocognitive issues
73% intellectual disability
93% SENs
92% tubers
80% angiomyolipomas
LAM
> 98% skin lesions
> 50% cardiac rhabdomyomas

Less severe
Mosaicism or
TSC1 or missense* TSC2 mutation
<50% seizures
Seizures easy to control
No autism/neurocognitive issues
No intellectual disability
few SENs, few tubers
70% angiomyolipomas
less LAM
Asymmetric facial angiofibroma
Few white spots, Shagreen patch
Few cardiac rhabdomyomas

Kwiatkowski lab

Current/past members
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Heng Du
Barbara Ogorek
Kathryn Lasseter
Katarzyna Klonowska
Yan Tang
Amin Nassar
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Mount Hancock Aug 2018

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