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

Klonowska et al.
Annu Rev Genomics Hum Genet. 2019

N=1638
TSC2 mutation spectrum – C half

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.
Mosaicism is common both in general and in TSC
RESEARCH ARTICLE

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

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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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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
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
Mosaicism detection in blood

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

• More readily done with DNA extracted directly from skin biopsy
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.
    

- 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%.
    
Phenotype

- Early Onset AF (<5y) or UF (<15y)
- ≥3 Mucocutaneous Findings

Genetic Workup

- Routine Blood Test
- NGS Blood
- NGS Skin Tumor

- Pathogenic Variant Detected
- Germline (VAF ≥ 40%)
- Mosaic (VAF < 40%)

Key:
- Germline
- Mosaic

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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Analysis of clinical features of 39 mosaic TSC individuals have a distinct clinical picture from those with classic TSC.
Mosaicism for a \textit{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

Mosaic mutant allele frequencies

8 infants

Mosaic mutant allele frequencies

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

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